



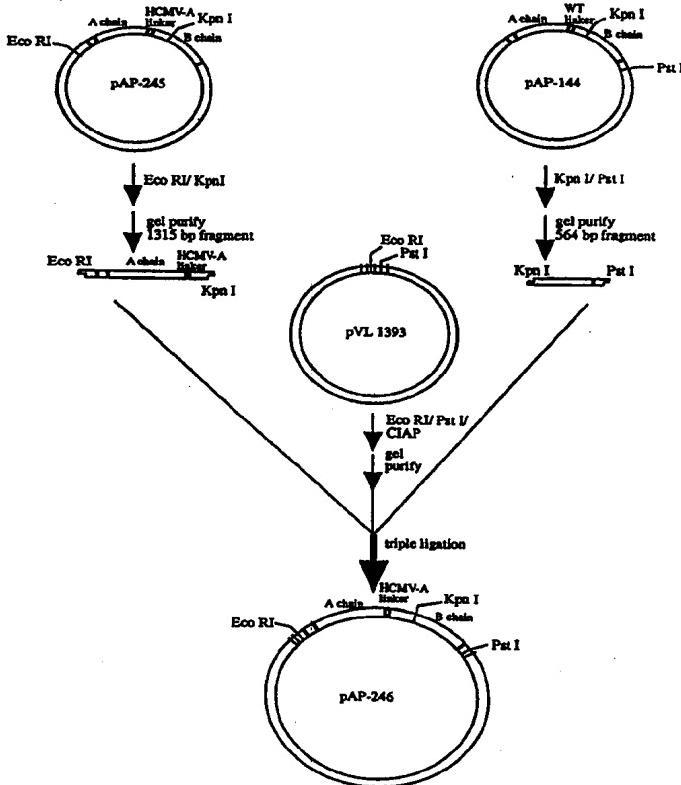
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## (54) Title: RICIN-LIKE TOXIN VARIANTS FOR TREATMENT OF CANCER, VIRAL OR PARASITIC INFECTIONS

## (57) Abstract

The present invention provides a protein having an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence, linking the A and B chains. The linker sequence contains a cleavage recognition site for a disease specific protease such as a cancer, fungal, viral or parasitic protease. The invention also relates to a nucleic acid molecule encoding the protein and to expression vectors incorporating the nucleic acid molecule. Also provided is a method of inhibiting or destroying mammalian cancer cells, cells infected with a virus, a fungus, or parasite, or parasites utilizing the nucleic acid molecules and proteins of the invention and pharmaceutical compositions for treating human cancer, viral infection, fungal infection, or parasitic infection.



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- 1 -

**Title: RICIN-LIKE TOXIN VARIANTS FOR TREATMENT OF CANCER, VIRAL OR PARASITIC INFECTIONS**

**FIELD OF THE INVENTION**

The invention relates to proteins useful as therapeutics  
5 against cancer, viral infections, parasitic and fungal infections. The  
proteins contain A and B chains of a ricin-like toxin linked by a linker  
sequence that is specifically cleaved and activated by proteases specific to  
disease-associated pathogens or cells.

**BACKGROUND OF THE INVENTION**

10 Bacteria and plants are known to produce cytotoxic  
proteins which may consist of one, two or several polypeptides or  
subunits. Those proteins having a single subunit may be loosely  
classified as Type I proteins. Many of the cytotoxins which have  
evolved two subunit structures are referred to as type II proteins  
15 (Saelinger, C.B. in Trafficking of Bacterial Toxins (eds. Saelinger, C.B.)  
1-13 (CRC Press Inc., Boca Raton, Florida, 1990). One subunit, the A  
chain, possesses the toxic activity whereas the second subunit, the B  
chain, binds cell surfaces and mediates entry of the toxin into a target  
cell. A subset of these toxins kill target cells by inhibiting protein  
20 biosynthesis. For example, bacterial toxins such as diphtheria toxin or  
Pseudomonas exotoxin inhibit protein synthesis by inactivating  
elongation factor 2. Plant toxins such as ricin, abrin, and bacterial toxin  
Shiga toxin, inhibit protein synthesis by directly inactivating the  
ribosomes (Olsnes, S. & Phil, A. in Molecular action of toxins and  
25 viruses (eds. Cohen, P. & vanHeyningen, S.) 51-105 Elsevier Biomedical  
Press, Amsterdam, 1982).

Ricin, derived from the seeds of *Ricinus communis*  
(castor oil plant), may be the most potent of the plant toxins. It is  
estimated that a single ricin A chain is able to inactivate ribosomes at a

- 2 -

rate of 1500 ribosomes/minute. Consequently, a single molecule of ricin is enough to kill a cell (Olsnes, S. & Phil, A. in Molecular action of toxins and viruses (eds. Cohen, P. & vanHeyningen, S.) (Elsevier Biomedical Press, Amsterdam, 1982). The ricin toxin is a glycosylated heterodimer consisting of A and B chains with molecular masses of 30,625 Da and 31,431 Da linked by a disulphide bond. The A chain of ricin has an N-glycosidase activity and catalyzes the excision of a specific adenine residue from the 28S rRNA of eukaryotic ribosomes (Endo, Y. & Tsurugi, K. J., *Biol. Chem.* 262:8128 (1987)). The B chain of ricin, although not toxic in itself, promotes the toxicity of the A chain by binding to galactose residues on the surface of eukaryotic cells and stimulating receptor-mediated endocytosis of the toxin molecule (Simmons et al., *Biol. Chem.* 261:7912 (1986)). Once the toxin molecule consisting of the A and B chains is internalized into the cell via clathrin-dependent or independent mechanisms, the greater reduction potential within the cell induces a release of the active A chain, eliciting its inhibitory effect on protein synthesis and its cytotoxicity (Emmanuel, F. et al., *Anal. Biochem.* 173: 134-141 (1988); Blum, J.S. et al., *J. Biol. Chem.* 266: 22091-22095 (1991); Fiani, M.L. et al., *Arch. Biochem. Biophys.* 307: 225-230 (1993)). Empirical evidence suggests that activated toxin (e.g. ricin, shiga toxin and others) in the endosomes is transcytosed through the trans-Golgi network to the endoplasmic reticulum by retrograde transport before the A chain is translocated into the cytoplasm to elicit its action (Sandvig, K. & van Deurs, B., *FEBS Lett.* 346: 99-102 (1994)).

Protein toxins are initially produced in an inactive, precursor form. Ricin is initially produced as a single polypeptide (proricin) with a 35 amino acid N-terminal presequence and 12 amino acid linker between the A and B chains. The pre-sequence is removed during translocation of the ricin precursor into the endoplasmic reticulum (Lord, J.M., *Eur. J. Biochem.* 146:403-409 (1985) and Lord, J.M., *Eur. J. Biochem.* 146:411-416 (1985)). The proricin is then

- 3 -

translocated into specialized organelles called protein bodies where a plant protease cleaves the protein at a linker region between the A and B chains (Lord, J.M. et al., *FASAB Journal* 8:201-208 (1994)). The two chains, however, remain covalently attached by an interchain disulfide bond (cysteine 259 in the A chain to cysteine 4 in the B chain) and mature disulfide linked ricin is stored in protein bodies inside the plant cells. The A chain is inactive in proricin (O'Hare, M. et al., *FEBS Lett.* 273:200-204 (1990)) and it is inactive in the disulfide-linked mature ricin (Richardson, P.T. et al., *FEBS Lett.* 255:15-20 (1989)). The ribosomes of the castor bean plant are themselves susceptible to inactivation by ricin A chain; however, as there is no cell surface galactose to permit B chain recognition the A chain cannot re-enter the cell. The exact mechanism of A chain release and activation in target cell cytoplasm is not known (Lord, J.M. et al., *FASAB Journal* 8:201-208 (1994)). However, it is known that for activation to take place the disulfide bond between the A and B chains must be reduced and, hence, the linkage between subunits broken.

Diphtheria toxin is produced by *Corynebacterium diphtheriae* as a 535 amino acid polypeptide with a molecular weight of approximately 58kD (Greenfield, L. et al., *Proc. Natl. Acad. Sci. USA* 80:6853-6857 (1983); Pastan, I. et al., *Annu. Rev. Biochem.* 61:331-354 (1992); Collier, R.J. & Kandel, J., *J. Biol. Chem.* 246:1496-1503 (1971)). It is secreted as a single-chain polypeptide consisting of 2 functional domains. Similar to proricin, the N-terminal domain (A-chain) contains the cytotoxic moiety whereas the C-terminal domain (B-chain) is responsible for binding to the cells and facilitates toxin endocytosis. Conversely, the mechanism of cytotoxicity for diphtheria toxin is based on ADP-ribosylation of EF-2 thereby blocking protein synthesis and producing cell death. The 2 functional domains in diphtheria toxin are linked by an arginine-rich peptide sequence as well as a disulphide bond. Once the diphtheria toxin is internalized into the cell, the arginine-rich peptide linker is cleaved by trypsin-like enzymes and the

- 4 -

disulphide bond (Cys 186-201) is reduced. The cytotoxic domain is subsequently translocated into the cytosol substantially as described above for ricin and elicits ribosomal inhibition and cytotoxicity.

*Pseudomonas* exotoxin is also a 66kD single-chain toxin

5 protein secreted by *Pseudomonas aeruginosa* with a similar mechanism of cytotoxicity to that of diphtheria toxin (Pastan, I. et al., *Annu. Rev. Biochem.* 61:331-354 (1992); Ogata, M. et al., *J. Biol. Chem.* 267:25396-25401 (1992); Vagil, M.L. et al., *Infect. Immunol.* 16:353-361 (1977)).

10 *Pseudomonas* exotoxin consists of 3 conjoint functional domains. The first domain Ia (amino acids 1-252) is responsible for cell binding and toxin endocytosis, a second domain II (amino acids 253-364) is responsible for toxin translocation from the endocytic vesicle to the cytosol, and a third domain III (amino acids 400-613) is responsible for protein synthesis inhibition and cytotoxicity. After *Pseudomonas*

15 exotoxin enters the cell, the liberation of the cytotoxic domain is effected by both proteolytic cleavage of a polypeptide sequence in the second domain (near Arg 279) and the reduction of the disulphide bond (Cys 265-287) in the endocytic vesicles. In essence, the overall pathway to cytotoxicity is analogous to diphtheria toxin with the exception that the

20 toxin translocation domain in *Pseudomonas* exotoxin is structurally distinct.

Other toxins possessing distinct functional domains for cytotoxicity and cell binding/toxin translocation include abrin, modeccin and volvensin (Sandvig, K. et al., *Biochem. Soc. Trans.* 21:707-711 (1993)). Some toxins such as Shiga toxin and cholera toxin also have multiple polypeptide chains responsible for receptor binding and endocytosis.

The ricin gene has been cloned and sequenced, and the X-ray crystal structures of the A and B chains have been described

30 (Rutenber, E. et al. *Proteins* 10:240-250 (1991); Weston et al., *Mol. Bio.* 244:410-422, 1994; Lamb and Lord, *Eur. J. Biochem.* 14:265 (1985); Halling, K. et al. *Nucleic Acids Res.* 13:8019 (1985)). Similarly, the genes for

- 5 -

diphtheria toxin and *Pseudomonas* exotoxin have been cloned and sequenced, and the 3-dimensional structures of the toxin proteins have been elucidated and described (Columblatti, M. et al., *J. Biol. Chem.* 261:3030-3035 (1986); Allured, V.S. et al., *Proc. Natl. Acad. Sci. USA* 83:1320-1324 (1986); Gray, G.L. et al., *Proc. Natl. Acad. Sci. USA* 81:2645-2649 (1984); Greenfield, L. et al., *Proc. Natl. Acad. Sci. USA* 80:6853-6857 (1983); Collier, R.J. et al., *J. Biol. Chem.* 257:5283-5285 (1982)).

The potential of bacterial and plant toxins for inhibiting mammalian retroviruses, particularly acquired immunodeficiency syndrome (AIDS), has been investigated. Bacterial toxins such as *Pseudomonas* exotoxin-A and subunit A of diphtheria toxin; dual chain ribosomal inhibitory plant toxins such as ricin, and single chain ribosomal inhibitory proteins such as trichosanthin and pokeweed antiviral protein have been used for the elimination of HIV infected cells (Olson et al., *AIDS Res. and Human Retroviruses* 7:1025-1030 (1991)). The high toxicity of these toxins for mammalian cells, combined with a lack of specificity of action poses a major problem to the development of pharmaceuticals incorporating the toxins, such as immunotoxins.

Due to their extreme toxicity there has been much interest in making ricin-based immunotoxins as therapeutic agents for specifically destroying or inhibiting infected or tumourous cells or tissues (Vitetta et al., *Science* 238:1098-1104(1987)). An immunotoxin is a conjugate of a specific cell binding component, such as a monoclonal antibody or growth factor and the toxin in which the two protein components are covalently linked. Generally, the components are chemically coupled. However, the linkage may also be a peptide or disulfide bond. The antibody directs the toxin to cell types presenting a specific antigen thereby providing a specificity of action not possible with the natural toxin. Immunotoxins have been made both with the entire ricin molecule (i.e. both chains) and with the ricin A chain alone (Spooner et al., *Mol. Immunol.* 31:117-125, (1994)).

- 6 -

Immunotoxins made with the ricin dimer (IT-Rs) are more potent toxins than those made with only the A chain (IT-As). The increased toxicity of IT-Rs is thought to be attributed to the dual role of the B chains in binding to the cell surface and in translocating the A chain to the cytosolic compartment of the target cell (Vitetta et al., *Science* 238:1098-1104 (1987); Vitetta & Thorpe, *Seminars in Cell Biology* 2:47-58 (1991)). However, the presence of the B chain in these conjugates also promotes the entry of the immunotoxin into nontarget cells. Even small amounts of B chain may override the specificity of the cell-binding component as the B chain will bind nonspecifically to galactose associated with N-linked carbohydrates, which is present on most cells. IT-As are more specific and safer to use than IT-Rs. However, in the absence of the B chain the A chain has greatly reduced toxicity. Due to the reduced potency of IT-As as compared to IT-Rs, large doses of IT-As must be administered to patients. The large doses frequently cause immune responses and production of neutralizing antibodies in patients (Vitetta et al., *Science* 238:1098-1104 (1987)). IT-As and IT-Rs both suffer from reduced toxicity as the A chain is not released from the conjugate into the target cell cytoplasm.

A number of immunotoxins have been designed to recognize antigens on the surfaces of tumour cells and cells of the immune system (Pastan et al., *Annals New York Academy of Sciences* 758:345-353 (1995)). A major problem with the use of such immunotoxins is that the antibody component is its only targeting mechanism and the target antigen is often found on non-target cells (Vitetta et al., *Immunology Today* 14:252-259 (1993)). Also, the preparation of a suitable specific cell binding component may be problematic. For example, antigens specific for the target cell may not be available and many potential target cells and infective organisms can alter their antigenic make up rapidly to avoid immune recognition. In view of the extreme toxicity of proteins such as ricin, the lack of

specificity of the immunotoxins may severely limit their usefulness as therapeutics for the treatment of cancer and infectious diseases.

The insertion of intramolecular protease cleavage sites between the cytotoxic and cell-binding components of a toxin can mimic 5 the way that the natural toxin is activated. European patent application no. 466,222 describes the use of maize-derived pro-proteins which can be converted into active form by cleavage with extracellular blood enzymes such as factor Xa, thrombin or collagenase. Garred, O. et al. (*J. Biol. Chem.* 270:10817-10821 (1995)) documented the use of a ubiquitous 10 calcium-dependent serine protease, furin, to activate shiga toxin by cleavage of the trypsin-sensitive linkage between the cytotoxic A-chain and the pentamer of cell-binding B-units. Westby et al. (*Bioconjugate Chem.* 3:375-381 (1992)) documented fusion proteins which have a specific cell binding component and proricin with a protease sensitive 15 cleavage site specific for factor Xa within the linker sequence. O'Hare et al. (*FEBS Lett.* 273:200-204 (1990)) also described a recombinant fusion protein of RTA and staphylococcal protein A joined by a trypsin-sensitive cleavage site. In view of the ubiquitous nature of the extracellular proteases utilized in these approaches, such artificial 20 activation of the toxin precursor or immunotoxin does not confer a mechanism for intracellular toxin activation and the problems of target specificity and adverse immunological reactions to the cell-binding component of the immunotoxin remain.

In a variation of the approach of insertion of 25 intramolecular protease cleavage sites on proteins which combine a binding chain and a toxic chain, Leppla, S.H. et al. (*Bacterial Protein Toxins zbl.bakt.suppl.* 24:431-442 (1994)) suggest the replacement of the native cleavage site of the protective antigen (PA) produced by *Bacillus anthracis* with a cleavage site that is recognized by cells that contain a 30 particular protease. PA, recognizes, binds, and thereby assists in the internalization of lethal factor (LF) and edema toxin (ET). also produced by *Bacillus anthracis*. However, this approach is wholly dependent on

the availability of LF, or ET and PA all being localized to cells wherein the modified PA can be activated by the specific protease. It does not confer a mechanism for intracellular toxin activation and presents a problem of ensuring sufficient quantities of toxin for internalization in  
5 target cells.

The *in vitro* activation of a *Staphylococcus*-derived pore-forming toxin,  $\alpha$ -hemolysin by extracellular tumour-associated proteases has been documented (Panchel, R.G. et al., *Nature Biotechnology* 14:852-857 (1996)). Artificial activation of  $\alpha$ -hemolysin *in*  
10 *vitro* by said proteases was reported but the actual activity and utility of  $\alpha$ -hemolysin in the destruction of target cells were not demonstrated.

Hemolysin does not inhibit protein synthesis but is a heptameric transmembrane pore which acts as a channel to allow leakage of molecules up to 3 kD thereby disrupting the ionic balances of  
15 the living cell. The  $\alpha$ -hemolysin activation domain is likely located on the outside of the target cell (for activation by extracellular proteases). The triggering mechanism in the disclosed hemolysin precursor does not involve the intracellular proteolytic cleavage of 2 functionally distinct domains. Also, the proteases used for the  $\alpha$ -hemolysin  
20 activation are ubiquitously secreted extracellular proteases and toxin activation would not be confined to activation in the vicinity of diseased cells. Such widespread activation of the toxin does not confer target specificity and limits the usefulness of said  $\alpha$ -hemolysin toxin as therapeutics due to systemic toxicity.

25 A variety of proteases specifically associated with malignancy, viral infections and parasitic infections have been identified and described. For example, cathepsin is a family of serine, cysteine or aspartic endopeptidases and exopeptidases which has been implicated to play a primary role in cancer metastasis (Schwartz, M.K.,  
30 *Clin. Chim. Acta* 237:67-78 (1995); Spiess, E. et al., *J. Histochem.*

- 9 -

*Cytochem.* 42:917-929 (1994); Scarborough, P.E. et al., *Protein Sci.* 2:264-276 (1993); Sloane, B.F. et al., *Proc. Natl. Acad. Sci. USA* 83:2483-2487 (1986); Mikkelsen, T. et al., *J. Neurosurge* 83:285-290 (1995)). Matrix metalloproteinases (MMPs or matrixins) are zinc-dependent proteinases consisting of collagenases, matrilysin, stromelysins, gelatinases and macrophage elastase (Krane, S.M., *Ann. N.Y. Acad. Sci.* 732:1-10 (1994); Woessner, J.F., *Ann. N.Y. Acad. Sci.* 732:11-21 (1994); Carvalho, K. et al., *Biochem. Biophys. Res. Comm.* 191:172-179 (1993); Nakano, A. et al. *J. of Neurosurge*, 83:298-307 (1995); Peng, K-W, et al. *Human Gene Therapy*, 8:729-738 (1997); More, D.H. et al. *Gynaecologic Oncology*, 65:78-82 (1997)). These proteases are involved in pathological matrix remodeling. Under normal physiological conditions, regulation of matrixin activity is effected at the level of gene expression. Enzymatic activity is also controlled stringently by tissue inhibitors of metalloproteinases (TIMPs) (Murphy, G. et al., *Ann. N.Y. Acad. Sci.* 732:31-41 (1994)). The expression of MMP genes is reported to be activated in inflammatory disorders (e.g. rheumatoid arthritis) and malignancy.

In malaria, parasitic serine and aspartic proteases are involved in host erythrocyte invasion by the *Plasmodium* parasite and in hemoglobin catabolism by intraerythrocytic malaria (O'Dea, K.P. et al., *Mol. Biochem. Parasitol.* 72:111-119 (1995); Blackman, M.J. et al., *Mol. Biochem. Parasitol.* 62:103-114 (1993); Cooper, J.A. et al., *Mol. Biochem. Parasitol.* 56:151-160 (1992); Goldberg, D.E. et al., *J. Exp. Med.* 173:961-969 (1991)). *Schistosoma mansoni* is also a pathogenic parasite which causes schistosomiasis or bilharzia. Elastinolytic proteinases have been associated specifically with the virulence of this particular parasite (McKerrow, J.H. et al., *J. Biol. Chem.* 260:3703-3707 (1985)).

Welch, A.R. et al. (*Proc. Natl. Acad. Sci. USA* 88:10797-10800 (1991)) has described a series of viral proteases which are specifically associated with human cytomegalovirus, human herpesviruses, Epstein-Barr virus, varicella zoster virus-I. and

- 10 -

infectious laryngotracheitis virus. These proteases possess similar substrate specificity and play an integral role in viral scaffold protein restructuring in capsid assembly and virus maturation. Other viral proteases serving similar functions have also been documented for  
5 human T-cell leukemia virus (Blaha, I. et al., *FEBS Lett.* 309:389-393 (1992); Pettit, S.C. et al., *J. Biol. Chem.* 266:14539-14547 (1991)), hepatitis viruses (Hirowatari, Y. et al., *Anal. Biochem.* 225:113-120 (1995); Hirowatari, Y. et al., *Arch. Virol.* 133:349-356 (1993); Jewell, D.A. et al., *Biochemistry* 31:7862-7869 (1992)), poliomyelitis virus (Weidner, J.R. et  
10 al., *Arch. Biochem. Biophys.* 286:402-408 (1991)), and human rhinovirus (Long, A.C. et al., *FEBS Lett.* 258:75-78 (1989)).

*Candida* yeasts are dimorphic fungi which are responsible for a majority of opportunistic infections in AIDS patients (Holmberg, K. and Myer, R., *Scand. J. Infect. Dis.* 18:179-192 (1986)). Aspartic  
15 proteinases have been associated specifically with numerous virulent strains of *Candida* including *Candida albican*, *Candida tropicalis*, and *Candida parapsilosis* (Abad-Zapatero, C. et al., *Protein Sci.* 5:640-652 (1996); Cutfield, S.M. et al., *Biochemistry* 35:398-410 (1995); Ruchel, R. et al, *Zentralbl. Bakteriol. Mikrobiol Hyg. I Abt. Orig. A.* 255:537-548 (1983);  
20 Remold, H. et al., *Biochim. Biophys. Acta* 167:399-406 (1968)), and the levels of these enzymes have been correlated with the lethality of the strain (Schreiber, B. et al., *Diagn. Microbiol. Infect. Dis.* 3:1-5 (1985)).

#### SUMMARY OF THE INVENTION

The invention relates to novel recombinant toxic  
25 proteins which are specifically toxic to diseased cells but do not depend for their specificity of action on a specific cell binding component. The recombinant proteins of the invention have an A chain of a ricin-like toxin linked to a B chain by a synthetic linker sequence which may be cleaved specifically by a protease localised in cells or tissues affected by a  
30 specific disease to liberate the toxic A chain thereby selectively inhibiting or destroying the diseased cells or tissues. The term diseased

- 11 -

cells as used herein, includes cells affected by cancer, or infected by fungi, or viruses, including retroviruses, or parasites.

Toxin targeting using the recombinant toxic proteins of the invention takes advantage of the fact that many DNA viruses 5 exploit host cellular transport mechanisms to escape immunological destruction. This is achieved by enhancing the retrograde translocation of host major histocompatibility complex (MHC) type I molecules from the endoplasmic reticulum into the cytoplasm (Bonifacino, J.S., *Nature* 384: 405-406 (1996); Wiertz, E.J. et al., *Nature* 384: 432-438 (1996)). The 10 facilitation of retrograde transport in diseased cells by the virus can enhance the transcytosis and cytotoxicity of a recombinant toxic protein of the present invention thereby further reducing non-specific cytotoxicity and improving the overall safety of the product.

The recombinant toxic proteins of the present invention 15 may be used to treat diseases including various forms of cancer such as T- and B-cell lymphoproliferative diseases, ovarian cancer, pancreatic cancer, head and neck cancer, squamous cell carcinoma, gastrointestinal cancer, breast cancer, prostate cancer, non small cell lung cancer, malaria, and diverse viral disease states associated with infection with 20 human cytomegalovirus, hepatitis virus, herpes virus, human rhinovirus, infectious laryngotracheitis virus, poliomyelitis virus, or varicella zoster virus.

In one aspect, the present invention provides a purified and isolated nucleic acid having a nucleotide sequence encoding an A 25 chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence, linking the A and B chains. The linker sequence is not a native linker sequence of a ricin-like toxin, but rather a synthetic heterologous linker sequence containing a cleavage recognition site for a disease-specific protease. The A and or 30 the B chain may be those of ricin.

In an embodiment, of the invention the cleavage recognition site is the cleavage recognition site for a cancer-associated

- 12 -

protease. In particular embodiments, the linker amino acid sequence comprises SLLKSRMVPNFN or SLLIARRMPNFN cleaved by cathepsin B; SKLVQASASGVN or SSYLKASDAPDN cleaved by an Epstein-Barr virus protease; RPKPQQFFGLMN cleaved by MMP-3 (stromelysin);

5 SLRPLALWRSFN cleaved by MMP-7 (matrilysin); SPQGIAGQRNFN cleaved by MMP-9; DVDERDVRGFASFL cleaved by a thermolysin-like MMP; SLPLGLWAPNFN cleaved by matrix metalloproteinase 2(MMP-2) ; SLLIFRSWANFN cleaved by cathepsin L; SGVVIATVIVIT cleaved by cathepsin D; SLGPQGIWGQFN cleaved by matrix metalloproteinase

10 1(MMP-1); KKSPGRVVGGSV cleaved by urokinase-type plasminogen activator; PQGLLGAPGILG cleaved by membrane type 1 matrixmetalloproteinase (MT-MMP); HGPEGLRVGFYESDVMGRGHARLVHVEEPHT cleaved by stromelysin 3 (or MMP-11), thermolysin, fibroblast collagenase and stromelysin-1;

15 GPQGLAGQRGIV cleaved by matrix metalloproteinase 13 (collagenase-3); GGSGQRGRKALE cleaved by tissue-type plasminogen activator(tPA); SLSALLSSDIFN cleaved by human prostate-specific antigen; SLPRFKIIGGFN cleaved by kallikrein (hK3); SLLGIAVPGNFN cleaved by neutrophil elastase; and FFKNIVTPRTPP cleaved by calpain

20 (calcium activated neutral protease). The nucleic acid sequences for ricin A and B chains with each of the linker sequences are shown in Figures 2D, 35C, 3D, 4D, 5D, 6D, 16D, 17D, 34C, 36C , 37C, 38C , 39C, 40C, 41C, 42C , 43C, 44C, 45C, 46C and 47C, respectively.

In another embodiment, the cleavage recognition site is

25 the cleavage recognition site for a protease associated with the malaria parasite, *Plasmodium falciparum*. In particular embodiments, the linker amino acid sequence comprises QVVQLQNYDEED; LPIFGESEDNDE; QVVTGEAISVTM; ALERTFLSFPTN or KFQDMLNISQHQ. The nucleic nucleotide sequences for ricin A and B

30 chains with each of the linker sequences are shown in Figures 7D, 8D, 9D, 10D, and 11D.

- 13 -

- In a another embodiment, the cleavage recognition site is the cleavage recognition site for a viral protease. The linker sequences preferably comprise the sequence Y-X-Y-A-Z wherein X is valine or leucine, Y is a polar amino acid, and Z is serine, asparagine or valine.
- 5 In particular embodiments, the linker amino acid sequence comprises SGVVNASCRLAN or SSYVKASVSPEN cleaved by a human cytomegalovirus protease; SALVNASSAHVN or STYLQASEKFKN cleaved by a herpes simplex 1 virus protease; SSILNASVPNFN cleaved by a human herpes virus 6 protease; SQDVNAVEASSN or
- 10 SVYLQASTGYGN cleaved by a varicella zoster virus protease; or SKYLQANEVITN cleaved by an infectious laryngotracheitis virus protease. The nucleic nucleotide sequences for ricin A and B chains with each of the linker sequences are shown in Figures 12D, 13D, 14D, 15D, 18D, 19D, 20D, and 22D.
- 15 In another embodiment, the cleavage recognition site is the cleavage recognition site for a hepatitis A viral protease. In particular embodiments, the linker amino acid sequence comprises SELRTQSFSNWN or SELWSQGIDDDN cleaved by a hepatitis A virus protease. The nucleic nucleotide sequences for ricin A and B chains with each of the linker sequences are shown in Figures 23D or 24D.
- 20 In another embodiment, the cleavage recognition site is the cleavage recognition site for a hepatitis C viral protease. In particular embodiments, the linker amino acid sequence comprises DLEVVTSTWVFN, DEMEECASHLFN, EDVVCCSMSYFN or
- 25 KGWRLLAPITAY cleaved by a hepatitis C virus protease. The nucleic nucleotide sequences for ricin A and B chains with each of the linker sequences are shown in Figures 30C, 31C, 32C and 33C.
- 30 In another embodiment, the cleavage recognition site is the cleavage recognition site for a *Candida* fungal protease. In particular embodiments, the linker amino acid sequence is SKPAKFRLNFn, SKPIEFFRLNFn or SKPAEFFALNFn cleaved by *Candida* aspartic

- 14 -

protease. The nucleic nucleotide sequences for ricin A and B chains with the first linker sequence are shown in Figures 25D.

The present invention also provides a plasmid incorporating the nucleic acid of the invention. In an embodiment, the 5 plasmid has the restriction map as shown in Figures 2A, 3A, 4A, 5A, 6A, 7A, 8A, 9A, 10A, 11A, 12A, 13A, 14A, 15A, 16A, 17A, 18A, 19A, 20A, 21A, 22A, 23A, 24A, or 25A.

In another embodiment, the present invention provides 10 a baculovirus transfer vector incorporating the nucleic acid of the invention. In particular embodiments, the invention provides a baculovirus transfer vector having the DNA sequence as shown in Figure 1.

In a further embodiment, the present invention provides 15 a baculovirus transfer vector incorporating the nucleic acid of the invention. In particular embodiments, the invention provides a baculovirus transfer vector having the restriction map as shown in Figures 2C, 3C, 4C, 5C, 6C, 7C, 8C, 9C, 10C, 11C, 12C, 13C, 14C, 15C, 16C, 17C, 18C, 19C, 20C, 21C, 22C, 23C, 24C, 25C, 30A, 31A, 32A, 33A, 34A, 35A, 20 36A, 37A, 38A, 39A, 40A, 41A, 42A, 43A, 44A, 45A, 46A, or 47A. or having the DNA sequence as shown in Figure 1.

In a further aspect, the present invention provides a recombinant protein comprising an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence, linking the A and B chains, wherein the linker sequence 25 contains a cleavage recognition site for a disease-specific protease (e.g.. a cancer, viral, parasitic, or fungal protease). The A and/or the B chain may be those of ricin. In an embodiment, the cleavage recognition site is the cleavage recognition site for a cancer, viral or parasitic protease substantially as described above. In a particular embodiment, the cancer 30 is T-cell or B-cell lymphoproliferative disease. In another particular embodiment, the virus is human cytomegalovirus, Epstein-Barr virus, hepatitis virus, herpes virus, human rhinovirus, infectious

- 15 -

laryngotracheitis virus, poliomyelitis virus, or varicella zoster virus. In a further particular embodiment, the parasite is *Plasmodium falciparum*.

In a further aspect, the invention provides a pharmaceutical composition for treating a fungal infection, such as *Candida*, in a mammal comprising the recombinant protein of the invention and a pharmaceutically acceptable carrier, diluent or excipient.

In yet another aspect, the invention provides a method of inhibiting or destroying cells affected by a disease, which cells are associated with a disease specific protease, including cancer or infection with a virus, fungus, or a parasite each of which has a specific protease, comprising the steps of preparing a recombinant protein of the invention having a heterologous linker sequence which contains a cleavage recognition site for the disease-specific protease and administering the recombinant protein to the cells. In an embodiment, the cancer is T-cell or B-cell lymphoproliferative disease, ovarian cancer, pancreatic cancer, head and neck cancer, squamous cell carcinoma, gastrointestinal cancer, breast cancer, prostate cancer, non small cell lung cancer. In another embodiment, the virus is human cytomegalovirus, Epstein-Barr virus, hepatitis virus, herpes virus, human rhinovirus, human T-cell leukemia virus, infectious laryngotracheitis virus, poliomyelitis virus, or varicella zoster virus. In another embodiment, the parasite is *Plasmodium falciparum*.

The present invention also relates to a method of treating a mammal with disease wherein cells affected by the disease are associated with a disease specific protease, including cancer or infection with a virus, fungus, or a parasite each of which has a specific protease by administering an effective amount of one or more recombinant proteins of the invention to said mammal.

Still further, a process is provided for preparing a pharmaceutical for treating a mammal with disease wherein cells

- 16 -

affected by the disease are associated with a disease specific protease, including cancer or infection with a virus, fungus, or a parasite each of which has a specific protease comprising the steps of preparing a purified and isolated nucleic acid having a nucleotide sequence  
5 encoding an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence, linking the A and B chains, wherein the linker sequence contains a cleavage recognition site for the disease-specific protease; introducing the nucleic acid into a host cell; expressing the nucleic acid in the host cell to obtain a recombinant  
10 protein comprising an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence, linking the A and B chains wherein the linker sequence contains the cleavage recognition site for the disease-specific protease; and suspending the protein in a pharmaceutically acceptable carrier, diluent or excipient.

15 In an embodiment, a process is provided for preparing a pharmaceutical for treating a mammal with disease wherein cells affected by the disease are associated with a disease specific protease, including cancer or infection with a virus, fungus, or a parasite each of which has a specific protease comprising the steps of identifying a  
20 cleavage recognition site for the protease; preparing a recombinant protein comprising an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence, linking the A and B chains wherein the linker sequence contains the cleavage recognition site for the protease and suspending the protein in a  
25 pharmaceutically acceptable carrier, diluent or excipient.

In a further aspect, the invention provides a pharmaceutical composition for treating for treating a mammal with disease wherein cells affected by the disease are associated with a disease specific protease, including cancer or infection with a virus, fungus, or a  
30 parasite comprising the recombinant protein of the invention and a pharmaceutically acceptable carrier, diluent or excipient.

Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

#### **DESCRIPTION OF THE DRAWINGS**

The invention will be better understood with reference to 10 the drawings in which:

Figure 1 shows the DNA sequence of the baculovirus transfer vector, pVL1393;

Figure 2A summarizes the cloning strategy used to generate the pAP-213 construct;

15 Figure 2B shows the nucleotide sequence of the Cathepsin B linker regions of pAP-213;

Figure 2C shows the subcloning of the Cathepsin B linker variant into a baculovirus transfer vector;

20 Figure 2D shows the DNA sequence of the pAP-214 insert containing ricin and the Cathepsin B linker;

Figure 3A summarizes the cloning strategy used to generate the pAP-215 construct;

Figure 3B shows the nucleotide sequence of the MMP-3 linker regions of pAP-215;

25 Figure 3C shows the subcloning of the MMP-3 linker variant into a baculovirus transfer vector;

Figure 3D shows the DNA sequence of the pAP-216 insert containing ricin and the MMP-3 linker;

30 Figure 4A summarizes the cloning strategy used to generate the pAP-217 construct;

Figure 4B shows the nucleotide sequence of the MMP-7 linker regions of pAP-217;

- 18 -

Figure 4C shows the subcloning of the MMP-7 linker variant into a baculovirus transfer vector;

Figure 4D shows the DNA sequence of the pAP-218 insert containing ricin and the MMP-7 linker;

5       Figure 5A summarizes the cloning strategy used to generate the pAP-219 construct;

Figure 5B shows the nucleotide sequence of the MMP-9 linker regions of pAP-219;

10      Figure 5C shows the subcloning of the MMP-9 linker variant into a baculovirus transfer vector;

Figure 5D shows the DNA sequence of the pAP-220 insert containing ricin and the MMP-9 linker.

Figure 6A summarizes the cloning strategy used to generate the pAP-221 construct;

15      Figure 6B shows the nucleotide sequence of the thermolysin-like MMP linker regions of pAP-221;

Figure 6C shows the subcloning of the thermolysin-like MMP linker variant into a baculovirus transfer vector.

20      Figure 6D shows the DNA sequence of the pAP-222 insert containing ricin and the thermolysin-like MMP linker;

Figure 7A summarizes the cloning strategy used to generate the pAP-223 construct;

Figure 7B shows the nucleotide sequence of the Plasmodium falciparum-A linker regions of pAP-223;

25      Figure 7C shows the subcloning of the Plasmodium falciparum-A linker variant into a baculovirus transfer vector;

Figure 7D shows the DNA sequence of the pAP-224 insert containing ricin and the Plasmodium falciparum-A linker;

30      Figure 8A summarizes the cloning strategy used to generate the pAP-225 construct;

Figure 8B shows the nucleotide sequence of the Plasmodium falciparum-B linker regions of pAP-225;

- 19 -

Figure 8C shows the subcloning of the Plasmodium falciparum-B linker variant into a baculovirus transfer vector;

Figure 8D shows the DNA sequence of the pAP-226 insert containing ricin and the Plasmodium falciparum-B linker;

5 Figure 9A summarizes the cloning strategy used to generate the pAP-227 construct;

Figure 9B shows the nucleotide sequence of the Plasmodium falciparum-C linker regions of pAP-227;

10 Figure 9C shows the subcloning of the Plasmodium falciparum-C linker variant into a baculovirus transfer vector;

Figure 9D shows the DNA sequence of the pAP-228 insert containing ricin and the Plasmodium falciparum-C linker;

Figure 10A summarizes the cloning strategy used to generate the pAP-229 construct;

15 Figure 10B shows the nucleotide sequence of the Plasmodium falciparum-D linker regions of pAP-229;

Figure 10C shows the subcloning of the Plasmodium falciparum-D linker variant into a baculovirus transfer vector;

20 Figure 10D shows the DNA sequence of the pAP-230 insert containing ricin and the Plasmodium falciparum-D linker;

Figure 11A summarizes the cloning strategy used to generate the pAP-231 construct;

Figure 11B shows the nucleotide sequence of the Plasmodium falciparum-E linker regions of pAP-231;

25 Figure 11C shows the subcloning of the Plasmodium falciparum-E linker variant into a baculovirus transfer vector;

Figure 11D shows the DNA sequence of the pAP-232 insert containing ricin and the Plasmodium falciparum-E linker;

30 Figure 12A summarizes the cloning strategy used to generate the pAP-233 construct;

Figure 12B shows the nucleotide sequence of the HSV-A linker regions of pAP-233;

- 20 -

Figure 12C shows the subcloning of the HSV-A linker variant into a baculovirus transfer vector;

Figure 12D shows the DNA sequence of the pAP-234 insert containing ricin and the HSV-A linker;

5       Figure 13A summarizes the cloning strategy used to generate the pAP-235 construct;

Figure 13B shows the nucleotide sequence of the HSV-B linker regions of pAP-235;

10      Figure 13C shows the subcloning of the HSV-B linker variant into a baculovirus transfer vector;

Figure 13D shows the DNA sequence of the pAP-236 insert containing ricin and the HSV-B linker;

Figure 14A summarizes the cloning strategy used to generate the pAP-237 construct;

15      Figure 14B shows the nucleotide sequence of the VZV-A linker regions of pAP-237;

Figure 14C shows the subcloning of the VZV-A linker variant into a baculovirus transfer vector;

20      Figure 14D shows the DNA sequence of the pAP-238 insert containing ricin and the VZV-A linker;

Figure 15A summarizes the cloning strategy used to generate the pAP-239 construct;

Figure 15B shows the nucleotide sequence of the VZV-B linker regions of pAP-239;

25      Figure 15C shows the subcloning of the VZV-B linker variant into a baculovirus transfer vector;

Figure 15D shows the DNA sequence of the pAP-240 insert containing ricin and the VZV-B linker;

30      Figure 16A summarizes the cloning strategy used to generate the pAP-241 construct;

Figure 16B shows the nucleotide sequence of the EBV-A linker regions of pAP-241;

- 21 -

Figure 16C shows the subcloning of the EBV-A linker variant into a baculovirus transfer vector;

Figure 16D shows the DNA sequence of the pAP-242 insert containing ricin and the EBV-A linker;

5       Figure 17A summarizes the cloning strategy used to generate the pAP-243 construct;

Figure 17B shows the nucleotide sequence of the EBV-B linker regions of pAP-243;

10      Figure 17C shows the subcloning of the EBV-B linker variant into a baculovirus transfer vector;

Figure 17D shows the DNA sequence of the pAP-244 insert containing ricin and the EBV-B linker;

Figure 18A summarizes the cloning strategy used to generate the pAP-245 construct;

15      Figure 18B shows the nucleotide sequence of the CMV-A linker regions of pAP-245;

Figure 18C shows the subcloning of the CMV-A linker variant into a baculovirus transfer vector;

20      Figure 18D shows the DNA sequence of the pAP-246 insert containing ricin and the CMV-A linker;

Figure 19A summarizes the cloning strategy used to generate the pAP-247 construct;

Figure 19B shows the nucleotide sequence of the CMV-B linker regions of pAP-247;

25      Figure 19C shows the subcloning of the CMV-B linker variant into a baculovirus transfer vector;

Figure 19D shows the DNA sequence of the pAP-248 insert containing ricin and the CMV-B linker.

30      Figure 20A summarizes the cloning strategy used to generate the pAP-249 construct;

Figure 20B shows the nucleotide sequence of the HHV-6 linker regions of pAP-249;

- 22 -

Figure 20C shows the subcloning of the HHV-6 linker variant into a baculovirus transfer vector;

Figure 20D shows the DNA sequence of the pAP-250 insert containing ricin and the HHV-6 linker;

5       Figure 21 shows the amino acid sequences of the wild type ricin linker and cancer protease-sensitive amino acid linkers contained in pAP-213 to pAP-222 and linkers pAP-241 to pAP-244;

Figure 22A summarizes the cloning strategy used to generate the pAP-253 construct;

10      Figure 22B shows the nucleotide sequence of the ILV linker regions of pAP-253;

Figure 22C shows the subcloning of the ILV linker variant into a baculovirus transfer vector;

15      Figure 22D shows the DNA sequence of the pAP-254 insert containing ricin and the ILV linker;

Figure 23A summarizes the cloning strategy used to generate the pAP-257 construct;

Figure 23B shows the nucleotide sequence of the HAV-A linker regions of pAP-257;

20      Figure 23C shows the subcloning of the HAV-A linker variant into a baculovirus transfer vector;

Figure 23D shows the DNA sequence of the pAP-258 insert containing ricin and the HAV-A linker;

25      Figure 24A summarizes the cloning strategy used to generate the pAP-255 construct;

Figure 24B shows the nucleotide sequence of the HAV-B linker regions of pAP-255;

Figure 24C shows the subcloning of the HAV-B linker variant into a baculovirus transfer vector;

30      Figure 24D shows the DNA sequence of the pAP-256 insert containing ricin and the HAV-B linker;

- 23 -

Figure 25A summarizes the cloning strategy used to generate the pAP-259 construct;

Figure 25B shows the nucleotide sequence of the CAN linker regions of pAP-259;

5 Figure 25C shows the subcloning of the CAN linker variant into a baculovirus transfer vector;

Figure 25D shows the DNA sequence of the pAP-260 insert containing ricin and the CAN linker;

10 Figure 26 shows the amino acid sequences of the wild type ricin linker and *Plasmodium falciparum* protease-sensitive amino acid linkers contained in linkers pAP-223 to pAP-232;

15 Figure 27 shows the amino acid sequences of the wild type ricin linker and the viral protease-sensitive amino acid linkers contained in pAP-233 to pAP-240, pAP-245-pAP-248, pAP-253 to pAP-258;

Figure 28 shows the amino acid sequences of the wild type ricin linker and the *Candida* aspartic protease-sensitive amino acid linker contained in pAP-259 to pAP-264;

20 Figure 29 describes an alternative mutagenesis and subcloning strategy to provide a baculovirus transfer vector containing the ricin-like toxin variant gene; and

Figure 30A summarizes the cloning strategy used to generate the pAP-262 construct;

25 Figure 30B shows the nucleotide sequence of the HCV-A linker region of pAP-262;

Figure 30C shows the DNA sequence of the pAP-262 insert;

Figure 30D shows the amino acid sequence comparison of mutant prororicin linker region HCV-A to wild type;

30 Figure 31A summarizes the cloning strategy used to generate the pAP-264 construct;

- 24 -

Figure 31B shows the nucleotide sequence of the HCV-B linker region of pAP-264;

Figure 31C shows the DNA sequence of the pAP-264 insert;

5 Figure 31D shows the amino acid sequence comparison of mutant prorocin linker region HCV-B to wild type;

Figure 32A summarizes the cloning strategy used to generate the pAP-266 construct;

10 Figure 32B shows the nucleotide sequence of the HCV-C linker region of pAP-266;

Figure 32C shows the DNA sequence of the pAP-266 insert;

Figure 32D shows the amino acid sequence comparison of mutant prorocin linker region HCV-C to wild type;

15 Figure 33A summarizes the cloning strategy used to generate the pAP-268 construct;

Figure 33B shows the nucleotide sequence of the HCV-D linker region of pAP-268;

20 Figure 33C shows the DNA sequence of the pAP-268 insert;

Figure 33D shows the amino acid sequence comparison of mutant prorocin linker region HCV-D to wild type;

Figure 34A summarizes the cloning strategy used to generate the pAP-270 construct;

25 Figure 34B shows the nucleotide sequence of the MMP-2 linker region of pAP-270;

Figure 34C shows the DNA sequence of the pAP-270 insert;

30 Figure 34D shows the amino acid sequence comparison of mutant prorocin linker region of MMP-2 to wild type;

Figure 35A summarizes the cloning strategy used to generate the pAP-272 construct;

- 25 -

Figure 35B shows the nucleotide sequence of the Cathepsin B (Site 2) linker region of pAP-272;

Figure 35C shows the DNA sequence of the pAP-272 insert;

5 Figure 35D shows the amino acid sequence comparison of mutant prororicin linker region of Cathepsin B (Site 2) to wild type;

Figure 36A summarizes the cloning strategy used to generate the pAP-274 construct;

10 Figure 36B shows the nucleotide sequence of the Cathepsin L linker region of pAP-274;

Figure 36C shows the DNA sequence of the pAP-274 insert;

Figure 36D shows the amino acid sequence comparison of mutant prororicin linker region of Cathepsin L to wild type;

15 Figure 37A summarizes the cloning strategy used to generate the pAP-276 construct;

Figure 37B shows the nucleotide sequence of the Cathepsin D linker region of pAP-276;

20 Figure 37C shows the DNA sequence of the pAP-276 insert;

Figure 37D shows the amino acid sequence comparison of mutant prororicin linker region of Cathepsin D to wild type;

Figure 38A summarizes the cloning strategy used to generate the pAP-278 construct;

25 Figure 38B shows the nucleotide sequence of the MMP-1 linker region of pAP-278;

Figure 38C shows the DNA sequence of the pAP-278 insert;

30 Figure 38D shows the amino acid sequence comparison of mutant prororicin linker region of MMP-1 to wild type;

Figure 39A summarizes the cloning strategy used to generate the pAP-280 construct;

- 26 -

Figure 39B shows the nucleotide sequence of the Urokinase-Type Plasminogen Activator linker region of pAP-280;

Figure 39C shows the DNA sequence of the pAP-280 insert;

5           Figure 39D shows the amino acid sequence comparison of mutant preroricin linker region of Urokinase-Type Plasminogen Activator to wild type;

Figure 40A summarizes the cloning strategy used to generate the pAP-282 construct;

10          Figure 40B shows the nucleotide sequence of the MT-MMP linker region of pAP-282;

Figure 40C shows the DNA sequence of the pAP-282 insert;

15          Figure 40D shows the amino acid sequence comparison of mutant preroricin linker region of MT-MMP to wild type;

Figure 41A summarizes the cloning strategy used to generate the pAP-284 construct;

Figure 41B shows the nucleotide sequence of the MMP-11 linker region of pAP-284;

20          Figure 41C shows the DNA sequence of the pAP-284 insert;

Figure 41D shows the amino acid sequence comparison of mutant preroricin linker region of MMP-11 to wild type;

25          Figure 42A summarizes the cloning strategy used to generate the pAP-286 construct;

Figure 42B shows the nucleotide sequence of the MMP-13 linker region of pAP-286;

Figure 42C shows the DNA sequence of the pAP-286 insert;

30          Figure 42D shows the amino acid sequence comparison of mutant preroricin linker region of MMP-13 to wild type;

- 27 -

Figure 43A summarizes the cloning strategy used to generate the pAP-288 construct;

Figure 43B shows the nucleotide sequence of the Tissue-type Plasminogen Activator linker region of pAP-288;

5           Figure 43C shows the DNA sequence of the pAP-288 insert;

Figure 43D shows the amino acid sequence comparison of mutant preproricin linker region of Tissue-type Plasminogen Activator to wild type;

10           Figure 44A summarizes the cloning strategy used to generate the pAP-290 construct;

Figure 44B shows the nucleotide sequence of the human Prostate-Specific Antigen linker region of pAP-290;

15           Figure 44C shows the DNA sequence of the pAP-290 insert;

Figure 44D shows the amino acid sequence comparison of mutant preproricin linker region of the human Prostate-Specific Antigen to wild type;

20           Figure 45A summarizes the cloning strategy used to generate the pAP-292 construct;

Figure 45B shows the nucleotide sequence of the kallikrein linker region of pAP-292;

Figure 45C shows the DNA sequence of the pAP-292 insert;

25           Figure 45D shows the amino acid sequence comparison of mutant preproricin linker region of the kallikrein to wild type;

Figure 46A summarizes the cloning strategy used to generate the pAP-294 construct;

30           Figure 46B shows the nucleotide sequence of the neutrophil elastase linker region of pAP-294;

Figure 46C shows the DNA sequence of the pAP-294 insert;

- 28 -

Figure 46D shows the amino acid sequence comparison of mutant prororicin linker region of neutrophil elastase to wild type;

Figure 47A summarizes the cloning strategy used to generate the pAP-296 construct;

5 Figure 47B shows the nucleotide sequence of the calpain linker region of pAP-296;

Figure 47C shows the DNA sequence of the pAP-296 insert;

10 Figure 47D shows the amino acid sequence comparison of mutant prororicin linker region of calpain to wild type;

Figure 48 is a blot showing cleavage of pAP-214 by Cathepsin B;

15 Figure 49 is a blot showing cleavage of pAP-220 with MMP-9;

Figure 50 is a blot showing activation of pAP-214; and

Figure 51 is a blot showing activation of pAP-220.

Figure 52 is a blot showing cleavage of pAP-248 with HCMV.

Figure 53 is a blot showing activation of pAP-248.

20 Figure 54 is a blot showing cleavage of pAP-256 by HAV 3C.

Figure 55 is a blot showing activation of pAP-256.

25 Figure 56 is a semi-logarithmic graph illustrating the cytotoxicity to COS-1 cells of undigested pAP-214 and pAP-214 digested with Cathepsin B.

Figure 57 is a semi-logarithmic graph illustrating the cytotoxicity of pAP-220 digested with MMP-9 compared to freshly thawed pAP-220 and ricin on COS-1 cells.

30 Figure 58 is a blot showing cleavage of pAP-270 with MMP-2.

Figure 59 is a blot showing activation of pAP-270.

Figure 60 is a blot showing cleavage of pAP-288 by t-PA.

- 29 -

Figure 61 is a blot showing activation of pAP-288.

Figure 62 is a blot showing cleavage of pAP-294 with human neutrophil elastase.

Figure 63 is a blot showing activation of pAP-294.

5       Figure 64 is a blot showing cleavage of pAP-296 with calpain.

Figure 65 is a blot showing activation of pAP-296.

Figure 66 is a blot showing cleavage of pAP-222 with MMP-2.

10      Figure 67 is a blot showing activation of pAP-222.

#### **DETAILED DESCRIPTION OF THE INVENTION**

##### **Nucleic Acid Molecules of the Invention**

As mentioned above, the present invention relates to novel nucleic acid molecules comprising a nucleotide sequence 15 encoding an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence, linking the A and B chains. The heterologous linker sequence contains a cleavage recognition site for a disease-specific protease (e.g. a viral protease, parasitic protease, cancer-associated protease, or a fungal protease).

20      The term "isolated and purified" as used herein refers to a nucleic acid substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors, or other chemicals when chemically synthesized. An "isolated and purified" nucleic acid is also substantially free of 25 sequences which naturally flank the nucleic acid (*i.e.* sequences located at the 5' and 3' ends of the nucleic acid) from which the nucleic acid is derived. The term "nucleic acid" is intended to include DNA and RNA and can be either double stranded or single stranded.

The term "linker sequence" as used herein refers to an 30 internal amino acid sequence within the protein encoded by the nucleic acid molecule of the invention which contains residues linking the A and B chain so as to render the A chain incapable of exerting its toxic

- 30 -

effect, for example catalytically inhibiting translation of a eukaryotic ribosome. By heterologous is meant that the linker sequence is not a sequence native to the A or B chain of a ricin-like toxin or precursor thereof. However, preferably, the linker sequence may be of a similar 5 length to the linker sequence of a ricin-like toxin and should not interfere with the role of the B chain in cell binding and transport into the cytoplasm. When the linker sequence is cleaved the A chain becomes active or toxic.

The nucleic acid molecule of the invention is cloned by 10 subjecting a preproricin cDNA clone to site-directed mutagenesis in order to generate a series of variants differing only in the sequence between the A and B chains (linker region). Oligonucleotides, corresponding to the extreme 5' and 3' ends of the preproricin gene are synthesized and used to PCR amplify the gene. Using the cDNA 15 sequence for preproricin (Lamb et al., *Eur. J. Biochem.* 145:266-270 (1985)), several oligonucleotide primers are designed to flank the start and stop codons of the preproricin open reading frame.

The preproricin cDNA is amplified using the upstream primer Ricin-99 or Ricin-109 and the downstream primer Ricin1729C 20 with Vent DNA polymerase (New England Biolabs) using standard procedures (Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, (Cold Spring Harbor Laboratory Press, 1989)). The purified PCR fragment encoding the preproricin cDNA is then ligated into an Eco RI-digested pBluescript II SK plasmid (Stratagene), and is 25 used to transform competent XL1-Blue cells (Stratagene). The cloned PCR product containing the putative preproricin gene is confirmed by DNA sequencing of the entire cDNA clone . The sequences and location of oligonucleotide primers used for sequencing are shown in Table 1.

30 The preproricin cDNA clone is subjected to site directed mutagenesis in order to generate a series of variants differing only in the sequence between the A and B chains (linker region). The wild-type

preroricin linker region is replaced with the heterogenous linker sequences that are cleaved by the various disease-specific proteases as shown in Figures 21, 26, 27, 28, and Part D of Figures 30-47. Linker identification as used herein in connection with the sequences provided  
5 in these figures have been assigned the sequence ID numbers as discussed below.

The linker regions of the variants encode a cleavage recognition sequence for a disease-specific protease associated with for example, cancer, viruses, parasites, or fungii. The mutagenesis and  
10 cloning strategy used to generate the disease-specific protease-sensitive linker variants are summarized in Part A of Figures 2-20, and Part A of Figures 22-25. The first step involves a DNA amplification using a set of mutagenic primers in combination with the two flanking primers Richin-99Eco or Ricin-109Eco and Ricin1729C Pst I. Restriction digested  
15 PCR fragments are gel purified and then ligated with PBluescript SK which has been digested with Eco RI and Pst I. Ligation reactions are used to transform competent XL1-Blue cells (Stratagene). Recombinant clones are identified by restriction digests of plasmid miniprep DNA and the mutant linker sequences are confirmed by DNA sequencing.  
20 With respect to the nucleotide sequences and amino acid sequences prepared as a result of the implementation of this strategy the following sequences have been assigned the sequence ID numbers as indicated.

SEQ ID NO. 1 is used herein in connection with the DNA sequence of the baculovirus transfer vector, pVL1393.

25 The nucleotide sequence of Cathepsin B linker regions of pAP-213 are referred to herein as SEQ ID NO. 2.

The nucleotide sequence of Cathepsin B linker regions of pAP-214 are referred to herein as SEQ ID NO. 3.

30 The nucleotide sequence of MMP-3 linker regions of pAP-215 are referred to herein as SEQ ID NO. 4.

The DNA sequence of the pAP-216 insert containing ricin and the MMP-3 linker are referred to herein as SEQ ID NO. 5.

- 32 -

The nucleotide sequence of MMP-7 linker regions of pAP-217 are referred to herein as SEQ ID NO. 6.

The DNA sequence of the pAP-218 insert containing ricin and the MMP-7 linker are referred to herein as SEQ ID NO. 7.

5 The nucleotide sequence of MMP-9 linker regions of pAP-219 are referred to herein as SEQ ID NO. 8.

The DNA sequence of the pAP-220 insert containing ricin and the MMP-9 are referred to herein as SEQ ID NO. 9.

10 The nucleotide sequence of thermolysin-like MMP linker regions of pAP-221 are referred to herein as SEQ ID NO. 10.

The DNA sequence of of pAP-222 insert containing ricin and the thermolysin-like MMP linker are referred to herein as SEQ ID NO. 11.

15 The nucleotide sequence of Plasmodium falciparum-A linker regions of pAP-223 are referred to herein as SEQ ID NO. 12.

The DNA sequence of the pAP-224 insert containing ricin and the Plasmodium falciparum-A linker are referred to herein as SEQ ID NO. 13.

20 The nucleotide sequence of Plasmodium falciparum-B linker regions of pAP-225 are referred to herein as SEQ ID NO. 14.

The DNA sequence of the pAP-226 insert containing ricin and the Plasmodium falciparum-B linker are referred to herein as SEQ ID NO. 15.

25 The nucleotide sequence of Plasmodium falciparum-C linker regions of pAP-227 are referred to herein as SEQ ID NO. 16.

The DNA sequence of the pAP-228 insert containing ricin and the Plasmodium falciparum-C linker are referred to herein as SEQ ID NO. 17.

30 The nucleotide sequence of the the Plasmodium falciparum-D linker regions of pAP-229 is referred to herein as SEQ ID NO. 18.

- 33 -

The DNA sequence of the pAP-230 insert containing ricin and the Plasmodium falciparum-D linker is referred to herein as SEQ ID NO. 19.

5 The nucleotide sequence of the Plasmodium falciparum-E linker regions of pAP-231 is referred to herein as SEQ ID NO. 20.

The DNA sequence of the pAP-232 insert containing ricin and the Plasmodium falciparum-E linker is referred to herein as SEQ ID NO. 21.

10 The nucleotide sequence of the HSV-A linker regions of pAP-233 is referred to herein as SEQ ID NO. 22.

The DNA sequence of the pAP-234 insert containing ricin and the HSV-A linker is referred to herein as SEQ ID NO. 23.

The nucleotide sequence of the HSV-B linker regions of pAP-235 is referred to herein as SEQ ID NO. 24.

15 The DNA sequence of the pAP-236 insert containing ricin and the HSV-B linker is referred to herein as SEQ ID NO. 25.

The nucleotide sequence of the VZV-A linker regions of pAP-237 are referred to herein as SEQ ID NO. 26.

20 The DNA sequence of the pAP-238 insert containing ricin and the VZV-A linker are referred to herein as SEQ ID NO. 27.

The nucleotide sequence of the VZV-B linker regions of PAP-239 is referred to herein as SEQ ID NO. 28.

The DNA sequence of the pAP-240 insert containing ricin and the VZV-B linker is referred to herein as SEQ ID NO. 29.

25 The nucleotide sequence of the EBV-A linker regions of pAP-241 is referred to herein as SEQ ID NO. 30.

The DNA sequence of the pAP-242 insert containing ricin and the EBV-A linker is referred to herein as SEQ ID NO. 31.

30 The nucleotide sequence of the EBV-B linker regions of pAP-243 is referred to herein as SEQ ID NO. 32.

The DNA sequence of the pAP-244 insert containing ricin and the EBV-B linker is referred to herein as SEQ ID NO. 33.

The nucleotide sequence of the CMV-A linker regions of pAP-245 is referred to herein as SEQ ID NO. 34.

The DNA sequence of the pAP-246 insert containing ricin and the CMV-A linker is referred to herein as SEQ ID NO. 35.

5 The nucleotide sequence of the CMV-B linker regions of pAP-247 is referred to herein as SEQ ID NO. 36.

The DNA sequence of the pAP-248 insert containing ricin and the CMV-B linker is referred to herein as SEQ ID NO. 37.

10 The nucleotide sequence of the HHV-6 linker regions of pAP-249 is referred to herein as SEQ ID NO. 38.

The DNA sequence of the pAP-250 insert containing ricin and the HHV-6 linker is referred to herein as SEQ ID NO. 39.

15 The amino acid sequences of the cancer protease-sensitive amino acid linkers contained in the following pAP proteins have the sequence ID numbers as indicated: pAP-213 and pAP-214 (SEQ ID NO. 40); pAP-215 and pAP-216 (SEQ ID NO. 41); pAP-217 and pAP-218; (SEQ ID NO. 42); pAP-219 and pAP-220 (SEQ ID NO. 43); and pAP-221 and pAP-222 (SEQ ID NO. 44).

20 The amino acid sequences of the following cancer protease-sensitive linkers are referred to herein with the corresponding sequence ID numbers: pAP-241 and pAP-242 (SEQ ID NO. 45); and pAP-243 and pAP-244 (SEQ ID NO. 46).

The nucleotide sequence of the ILV linker regions of pAP-253 is referred to herein as SEQ ID NO. 47.

25 The DNA sequence of the pAP-254 insert containing ricin and the ILV linker is referred to herein as SEQ ID NO. 48.

The nucleotide sequence of the HAV-A linker regions of pAP-257 is referred to herein as SEQ ID NO. 49.

30 The DNA sequence of the pAP-258 insert containing ricin and HAV-A linker is referred to herein as SEQ ID NO. 50.

The nucleotide sequence of the HAV-B linker regions of pAP-255 is referred to herein as SEQ ID NO. 51.

- 35 -

The DNA sequence of the pAP-256 insert containing ricin and the HAV-B linker is referred to herein as SEQ ID NO. 52.

The nucleotide sequence of the CAN linker regions of pAP-259 is referred to herein as SEQ ID NO. 53.

5 The DNA sequence of the pAP-260 insert containing ricin and the CAN linker is referred to herein as SEQ ID NO. 54.

The amino acid sequences of *Plasmodium falciparum* protease-sensitive linkers are referred to herein by the sequence ID numbers as follows: pAP-223 and pAP-224 (SEQ ID NO 55); pAP-225 and  
10 pAP-226 (SEQ ID NO 56); pAP-227 and pAP-228 (SEQ ID NO 57); pAP-229 and pAP-230 (SEQ ID NO 58); and pAP-231 and pAP-232 (SEQ ID NO 59) (see Figure 26).

15 The amino acid sequences of the viral protease-sensitive linkers which follow are referred to herein by the sequence ID numbers indicated: pAP-233 and pAP 234 (SEQ ID NO 60); pAP-235 and pAP-236 (SEQ ID NO 61); and pAP-249 and pAP-250 (SEQ ID NO 62) (see Figure 27).

20 The amino acid sequences of the viral protease-sensitive linkers which follow are referred to herein by the sequence ID numbers indicated: pAP-245 and pAP-246 (SEQ ID NO 63) ; and pAP-247 and pAP-248 (SEQ ID NO 64) (see Figure 27).

25 The amino acid sequences of the viral protease-sensitive linkers which follow are referred to herein by the sequence ID numbers indicated: pAP-237 and pAP-238 (SEQ ID NO 65); and pAP-239 and pAP-240 (SEQ ID NO 66); pAP-253 and pAP-254 (SEQ ID NO 67); pAP-255 and pAP-256 (SEQ ID NO 68); and pAP-257 and pAP-258 (SEQ ID NO 69) (see Figure 27).

30 The amino acid sequences of the *Candida* aspartic protease-sensitive linkers are referred to herein by the sequence ID numbers indicated: pAP-259 and pAP-260 (SEQ ID NO 70); pAP-261 and pAP-262 (SEQ ID NO 71); and pAP-263 and pAP-264 (SEQ ID NO 72 ).

- 36 -

An alternative mutagenesis and cloning strategy that can be used to generate the disease-specific protease-sensitive linker variants is summarized in Figure 29. The first step of this method involves a DNA amplification using a set of mutagenic primers in combination with the two flanking primers Ricin-109Eco and Ricin1729Pst. Restriction digested PCR fragments (Eco RI and Pst I) are gel purified. Preproricin variants produced from this method can be subcloned directly into the baculovirus transfer vector digested with Eco RI and Pst I and intermediate ligation steps involving pBluescript SK and pSB2 are circumvented. The cloning strategies used to generate disease-specific protease-sensitive linker variants are summarized in Part A of Figures 30 to 47. With respect to the nucleotide sequences and amino acid sequences prepared as a result of the implementation of this strategy the following sequences have been assigned the sequence ID numbers as indicated.

The nucleotide sequence of the HCV-A linker region of pAP-262 is referred to herein as SEQ ID NO. 73.

The DNA sequence of the pAP-262 insert is referred to herein as SEQ ID NO. 74.

The amino acid sequence of the mutant preproricin linker region for HCV-A, pAP-262, is referred to herein as SEQ ID NO. 75.

The nucleotide sequence of the HCV-B linker region of pAP-264 is referred to herein as SEQ ID NO. 76.

The DNA sequence of the pAP-264 insert is referred to herein as SEQ ID NO. 77.

The amino acid sequence of the mutant preproricin linker region for HCV-B, pAP-264, is referred to herein as SEQ ID NO. 78.

The nucleotide sequence of the HCV-C linker region of pAP-266 is referred to herein as SEQ ID NO. 79.

- 37 -

The DNA sequence of the pAP-266 insert is referred to herein as SEQ ID NO. 80.

The amino acid sequence of the mutant prorocin linker region for HCV-C, pAP-266, is referred to herein as SEQ ID NO.

5 81.

The nucleotide sequence of the HCV-D linker region of pAP-268 is referred to herein as SEQ ID NO. 82.

The DNA sequence of the pAP-268 insert is referred to herein as SEQ ID NO. 83.

10 The amino acid sequence of the mutant prorocin linker region for HCV-D , pAP-268, is referred to herein as SEQ ID NO. 84.

The nucleotide sequence of the MMP-2 linker region of pAP-270 is referred to herein as SEQ ID NO. 85.

15 The DNA sequence of the pAP-270 insert is referred to herein as SEQ ID NO. 86.

The amino acid sequence of the mutant prorocin linker region for MMP-2, pAP-270, is referred to herein as SEQ ID NO. 87.

20 The nucleotide acid sequence of the Cathepsin B (Site 2) linker region of pAP-272 is referred to herein as SEQ ID NO. 88.

The DNA sequence of the pAP-272 insert is referred to herein as SEQ ID NO. 89.

25 The amino acid sequence of the mutant prorocin linker region for Cathepsin B (Site 2), pAP-272, is referred to herein as SEQ ID NO. 90.

The nucleotide sequence of the Cathepsin L linker region of pAP-274 is referred to herein as SEQ ID NO. 91.

30 The DNA sequence of the pAP-274 insert is referred to herein as SEQ ID NO. 92.

- 38 -

The amino acid sequence of the mutant prororicin linker region of Cathepsin L, pAP-274, is referred to herein as SEQ ID NO. 93.

5 The nucleotide sequence of Cathepsin D linker region of pAP-276 is referred to herein as SEQ ID NO. 94.

The DNA sequence of the pAP-276 insert is referred to herein as SEQ ID NO. 95.

10 The amino acid sequence of the mutant prororicin linker region for Cathepsin D, pAP-276, is referred to herein as SEQ ID NO. 96.

The nucleotide sequence of the MMP-1 linker region of pAP-278 is referred to herein as SEQ ID NO. 97.

The DNA sequence of the pAP-278 insert is referred to herein as SEQ ID NO. 98.

15 The amino acid sequence of the mutant prororicin linker region for MMP-1, pAP-278, is referred to herein as SEQ ID NO. 99.

20 The nucleotide sequence of the Urokinase-Type Plasminogen Activator linker region of pAP-280 is referred to herein as SEQ ID NO. 100.

The DNA sequence of the pAP-280 insert is referred to herein as SEQ ID NO. 101.

25 The amino acid sequence of the mutant prororicin linker region for Urokinase-Type Plasminogen Activator, pAP-280, is referred to herein as SEQ ID NO. 102.

The nucleotide sequence of MT-MMP linker region of pAP-282 is referred to herein as SEQ ID NO. 103.

The DNA sequence of the pAP-282 insert is referred to herein as SEQ ID NO. 104.

30 The amino acid sequence of the mutant prororicin linker region for MT-MMP, pAP-282, is referred to herein as SEQ ID NO. 105.

- 39 -

The nucleotide sequence of the MMP-11 linker region of pAP-284 is referred to herein as SEQ ID NO. 106.

The DNA sequence of the pAP-284 insert is referred to herein as SEQ ID NO. 107.

5 The amino acid sequence of the mutant prorocin linker region for MMP-11, pAP-284, is referred to herein as SEQ ID NO. 108.

The nucleotide sequence of the MMP-13 linker region of pAP-286 is referred to herein as SEQ ID NO. 109.

10 The DNA sequence of the pAP-286 insert is referred to herein as SEQ ID NO. 110.

The amino acid sequence of the mutant prorocin linker region for MMP-13, pAP-286, is referred to herein as SEQ ID NO. 111.

15 The nucleotide sequence of the Tissue-type Plasminogen Activator linker region of pAP-288 is referred to herein as SEQ ID NO. 112.

The DNA sequence of the pAP-288 insert is referred to herein as SEQ ID NO. 113.

20 The amino acid sequence of the mutant prorocin linker region for Tissue-type Plasminogen Activator, pAP-288, is referred to herein as SEQ ID NO. 114.

25 The nucleotide sequence of the human Prostate-Specific Antigen linker region of pAP-290 is referred to herein as SEQ ID NO. 115.

The DNA sequence of the pAP-290 insert is referred to herein as SEQ ID NO. 116.

30 The amino acid sequence of the mutant prorocin linker region for the human Prostate-Specific Antigen, pAP-290, is referred to herein as SEQ ID NO. 117.

The nucleotide sequence of the kallikrein linker region of pAP-292 is referred to herein as SEQ ID NO. 118.

- 40 -

The DNA sequence of the pAP-292 insert is referred to herein as SEQ ID NO. 119.

5 The amino acid sequence of the mutant preproricin linker region for the kallikrein, pAP-292, is referred to herein as SEQ ID NO. 120.

The nucleotide sequence of the neutrophil elastase linker region of pAP-294 is referred to herein as SEQ ID NO. 121.

The DNA sequence of the pAP-294 insert is referred to herein as SEQ ID NO. 122.

10 The amino acid sequence of the mutant preproricin linker region for neutrophil elastase, pAP-294, is referred to herein as SEQ ID NO. 123.

The nucleotide sequence of the calpain linker region of pAP-296 is referred to herein as SEQ ID NO. 124.

15 The DNA sequence of the pAP-296 insert is referred to herein as SEQ ID NO. 125.

The amino acid sequence of the mutant preproricin linker region for calpain, pAP-296, is referred to herein as SEQ ID NO. 126.

20 The amino acid sequence of the wild type linker region is referred to herein as SEQ ID NO. 127.

The nucleic acid molecule of the invention has sequences encoding an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker sequence containing a cleavage recognition site for a disease-specific protease. The nucleic acid may be expressed to provide a recombinant protein having an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker sequence containing a cleavage recognition site for a disease-specific protease.

30 The nucleic acid molecule may comprise the A and/or B chain of ricin. The ricin gene has been cloned and sequenced, and the X-ray crystal structures of the A and B chains are published (Rutener, E., et al. *Proteins* 10:240-250 (1991); Weston et al., *Mol. Biol.* 244:410-422

- 41 -

(1994); Lamb and Lord, *Eur. J. Biochem.* 14:265 (1985); Halling, K., et al., *Nucleic Acids Res.* 13:8019 (1985)). It will be appreciated that the invention includes nucleic acid molecules encoding truncations of A and B chains of ricin like proteins and analogs and homologs of A and B chains of ricin-like proteins and truncations thereof (i.e., ricin-like proteins), as described herein. It will further be appreciated that variant forms of the nucleic acid molecules of the invention which arise by alternative splicing of an mRNA corresponding to a cDNA of the invention are encompassed by the invention.

Another aspect of the invention provides a nucleotide sequence which hybridizes under high stringency conditions to a nucleotide sequence encoding the A and/or B chains of a ricin-like protein. Appropriate stringency conditions which promote DNA hybridization are known to those skilled in the art, or can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1 6.3.6. For example, 6.0 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 x SSC at 50°C may be employed. The stringency may be selected based on the conditions used in the wash step. By way of example, the salt concentration in the wash step can be selected from a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be at high stringency conditions, at about 65°C.

The nucleic acid molecule may comprise the A and/or B chain of a ricin-like toxin. Methods for cloning ricin-like toxins are known in the art and are described, for example, in E.P. 466,222. Sequences encoding ricin or ricin-like A and B chains may be obtained by selective amplification of a coding region, using sets of degenerative primers or probes for selectively amplifying the coding region in a genomic or cDNA library. Appropriate primers may be selected from the nucleic acid sequence of A and B chains of ricin or ricin-like toxins. It is also possible to design synthetic oligonucleotide primers from the nucleotide sequences for use in PCR. Suitable primers may be selected

- 42 -

from the sequences encoding regions of ricin-like proteins which are highly conserved, as described for example in U.S. Patent No 5,101,025 and E.P. 466,222.

A nucleic acid can be amplified from cDNA or genomic DNA using these oligonucleotide primers and standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. It will be appreciated that cDNA may be prepared from mRNA, by isolating total cellular mRNA by a variety of techniques, for example, by 10 using the guanidinium-thiocyanate extraction procedure of Chirgwin et al., *Biochemistry* 18, 5294-5299 (1979). cDNA is then synthesized from the mRNA using reverse transcriptase (for example, Moloney MLV reverse transcriptase available from Gibco/BRL, Bethesda, MD, or AMV reverse transcriptase available from Seikagaku America, Inc., St. Petersburg, FL). It will be appreciated that the methods described above may be used to obtain the coding sequence from plants, bacteria or fungi, preferably plants, which produce known ricin-like proteins and also to screen for the presence of genes encoding as yet unknown ricin-like proteins.

20 A sequence containing a cleavage recognition site for a specific protease may be selected based on the disease or the pathogen which is to be targeted by the recombinant protein. The cleavage recognition site may be selected from sequences known to encode a cleavage recognition site for the cancer, viral or parasitic protease. 25 Sequences encoding cleavage recognition sites may be identified by testing the expression product of the sequence for susceptibility to cleavage by the respective protease.

A sequence containing a cleavage recognition site for a viral, fungal, parasitic or cancer associated protease may be selected 30 based on the retrovirus which is to be targeted by the recombinant protein. The cleavage recognition site may be selected from sequences known to encode a cleavage recognition site for the viral, fungal,

parasitic or cancer associated protease. Sequences encoding cleavage recognition sites may be identified by testing the expression product of the sequence for susceptibility to cleavage by a viral, fungal, parasitic or cancer associated protease. A polypeptide containing the suspected 5 cleavage recognition site may be incubated with a protease and the amount of cleavage product determined (DiLannit, 1990, *J. Biol. Chem.* 285: 17345-17354 (1990)).

The protease may be prepared by methods known in the art and used to test suspected cleavage recognition sites.

10 In one embodiment, the preparation of tumour-associated cathepsin B, its substrates and enzymatic activity assay methodology have been described by Sloane, B.F. et al. (*Proc. Natl. Acad. Sci. USA* 83:2483-2487 (1986)), Schwartz, M.K. (*Clin. Chim. Acta* 237:67-78 (1995)), and Panchal, R.G. et al. (*Nature Biotechnol.* 14:852-856 (1996)).  
15 The preparation of Epstein-Barr virus protease, its substrates and enzymatic activity assay methodology have been described by Welch, A.R. (*Proc. Natl. Acad. Sci. USA* 88:10792-10796 (1991)).

In another embodiment, the preparation of *Plasmodium falciparum* proteases, their substrates and enzymatic activity assay 20 methodology have been described by Goldberg, D.E. et al. (*J. Exp. Med.* 173:961-969 (1991)), Cooper & Bujard (*Mol. Biochem. Parasitol.* 56:151-160 (1992)), Nwagwu, M. et al. (*Exp. Parasitol.* 75:399-414 (1992)), Rosenthal, P.J. et al. (*J. Clin. Invest.* 91:1052-1056 (1993)), Blackman, M.J. et al. (*Mol. Biochem. Parasitol.* 62:103-114 (1995)).

25 In a further embodiment, the preparation of proteases from human cytomegalovirus, human herpes virus, varicella zoster virus and infectious laryngotracheitis virus have been taught by Liu F. & Roizman, B. (*J. Virol.* 65:5149-5156 (1991)) and Welch, A.R. (*Proc. Natl. Acad. Sci. USA* 88:10792-10796 (1991)). In addition, their respective 30 substrates and enzymatic activity assay methodologies are also described.

- 44 -

In another embodiment, the preparation of hepatitis A virus protease, its substrates and enzymatic activity assay methodology have been described by Jewell, D.A. et al. (*Biochemistry* 31:7862-7869 (1992)). The preparation of poliovirus protease, its substrates and 5 enzymatic activity assay methodology have been described by Weidner, J.R. et al. (*Arch. Biochem. Biophys.* 286:402-408 (1991)). The preparation of human rhinovirus protease, its substrates and enzymatic activity assay methodology have been described by Long, A.C. et al. (*FEBS Lett.* 258:75-78 (1989)).

10 In another embodiment of the invention, the preparation of proteases associated with *Candida* yeasts their substrates and enzymatic activity are contemplated, including the aspartic proteinases which have been associated specifically with numerous virulent strains of *Candida* including *Candida albican*, *Candida tropicalis*, and *Candida parapsilosis* (Abad-Zapatero, C. et al., *Protein Sci.* 5:640-652 (1996); Cutfield, S.M. et al., *Biochemistry* 35:398-410 (1995); Ruchel, R. et al., *Zentralbl. Bakteriol. Mikrobiol Hyg. I Abt. Orig. A.* 255:537-548 (1983); Remold, H. et al., *Biochim. Biophys. Acta* 167:399-406 (1968)).

20 The nucleic acid molecule of the invention may be prepared by site directed mutagenesis. For example, the cleavage site of a disease-specific protease may be prepared by site directed mutagenesis of the homologous linker sequence of a proricin-like toxin. Procedures for cloning proricin-like genes, encoding a linker sequence are described in EP 466,222. Site directed mutagenesis may be accomplished by DNA 25 amplification of mutagenic primers in combination with flanking primers. Suitable procedures using the mutagenic primers are shown in Parts A and B of Figures 1-4, Figures 13-16, Figures 18-36, Figures 38-41, and Figures 50-67.

30 The nucleic acid molecule of the invention may also encode a fusion protein. A sequence encoding a heterologous linker sequence containing a cleavage recognition site for a disease-specific protease may be cloned from a cDNA or genomic library or chemically

synthesized based on the known sequence of such cleavage sites. The heterologous linker sequence may then be fused in frame with the sequences encoding the A and B chains of the ricin-like toxin for expression as a fusion protein. It will be appreciated that a nucleic acid  
5 molecule encoding a fusion protein may contain a sequence encoding an A chain and a B chain from the same ricin-like toxin or the encoded A and B chains may be from different toxins. For example, the A chain may be derived from ricin and the B chain may be derived from abrin. A protein may also be prepared by chemical conjugation of the A and B  
10 chains and linker sequence using conventional coupling agents for covalent attachment.

An isolated and purified nucleic acid molecule of the invention which is RNA can be isolated by cloning a cDNA encoding an A and B chain and a linker into an appropriate vector which allows  
15 for transcription of the cDNA to produce an RNA molecule which encodes a protein of the invention. For example, a cDNA can be cloned downstream of a bacteriophage promoter, (e.g. a T7 promoter) in a vector, cDNA can be transcribed in vitro with T7 polymerase, and the resultant RNA can be isolated by standard techniques.

## 20 Recombinant Protein of the Invention

As previously mentioned, the invention provides novel recombinant proteins which incorporate the A and B chains of a ricin like toxin linked by a heterologous linker sequence containing a cleavage recognition site for a disease-specific protease. It is an  
25 advantage of the recombinant proteins of the invention that they are non-toxic until the A chain is liberated from the B chain by specific cleavage of the linker by the target protease.

Thus the protein may be used to specifically target cancer cells or cells infected with a virus or parasite in the absence of additional  
30 specific cell-binding components to target infected cells. It is a further advantage that the disease-specific protease cleaves the heterologous linker intracellularly thereby releasing the toxic A chain directly into

the cytoplasm of the cancer cell or infected cell. As a result, said cells are specifically targeted and non-infected normal cells are not directly exposed to the activated free A chain.

Ricin is a plant derived ribosome inhibiting protein  
5 which blocks protein synthesis in eukaryotic cells. Ricin may be derived from the seeds of *Ricinus communis* (castor oil plant). The ricin toxin is a glycosylated heterodimer with A and B chain molecular masses of 30,625 Da and 31,431 Da respectively. The A chain of ricin has an N-glycosidase activity and catalyzes the excision of a specific adenine  
10 residue from the 28S rRNA of eukaryotic ribosomes (Endo, Y; & Tsurugi, K. J. Biol. Chem. 262:8128 (1987)). The B chain of ricin, although not toxic in itself, promotes the toxicity of the A chain by binding to galactose residues on the surface of eukaryotic cells and stimulating receptor-mediated endocytosis of the toxin molecule  
15 (Simmons et al., *Biol. Chem.* 261:7912 (1986)).

All protein toxins are initially produced in an inactive, precursor form. Ricin is initially produced as a single polypeptide (preproricin) with a 35 amino acid N-terminal presequence and 12 amino acid linker between the A and B chains. The pre-sequence is  
20 removed during translocation of the ricin precursor into the endoplasmic reticulum (Lord, J.M., *Eur. J. Biochem.* 146:403-409 (1985) and Lord, J.M., *Eur. J. Biochem.* 146:411-416 (1985)). The proricin is then translocated into specialized organelles called protein bodies where a plant protease cleaves the protein at a linker region between the A and  
25 B chains (Lord, J.M. et al., *FASAB Journal* 8:201-208 (1994)). The two chains, however, remain covalently attached by an interchain disulfide bond (cysteine 259 in the A chain to cysteine 4 in the B chain) and mature disulfide linked ricin is stored in protein bodies inside plant cells. The A chain is inactive in the proricin (O'Hare, M., et al., *FEBS Lett.* 273:200-204 (1990)) and it is inactive in the disulfide-linked mature ricin (Richardson, P.T. et al., *FEBS Lett.* 255:15-20 (1989)). The ribosomes of the castor bean plant are themselves susceptible to inactivation by  
30

ricin A chain; however, as there is no cell surface galactose to permit B chain recognition the A chain cannot re-enter the cell.

Ricin-like proteins include, but are not limited to, bacterial, fungal and plant toxins which have A and B chains and 5 inactivate ribosomes and inhibit protein synthesis. The A chain is an active polypeptide subunit which is responsible for the pharmacologic effect of the toxin. In most cases the active component of the A chain is an enzyme. The B chain is responsible for binding the toxin to the cell surface and is thought to facilitate entry of the A chain into the cell 10 cytoplasm. The A and B chains in the mature toxins are linked by disulfide bonds. The toxins most similar in structure to ricin are plant toxins which have one A chain and one B chain. Examples of such toxins include abrin which may be isolated from the seeds of *Abrus precatorius* and modeccin.

15 Ricin-like bacterial proteins include diphtheria toxin, which is produced by *Corynebacterium diphtheriae*, *Pseudomonas enterotoxin* A and cholera toxin. It will be appreciated that the term ricin-like toxins is also intended to include the A chain of those toxins which have only an A chain. The recombinant proteins of the 20 invention could include the A chain of these toxins conjugated to, or expressed as, a recombinant protein with the B chain of another toxin. Examples of plant toxins having only an A chain include trichosanthin, MMC and pokeweed antiviral proteins, dianthin 30, dianthin 32, crotin II, curcin II and wheat germ inhibitor. Examples of fungal toxins 25 having only an A chain include alpha-sarcin, restrictocin, mitogillin, enomycin, phenomycin. Examples of bacterial toxins having only an A chain include cytotoxin from *Shigella dysenteriae* and related Shiga-like toxins. Recombinant trichosanthin and the coding sequence thereof is disclosed in U.S. Patents 5,101,025 and 5,128,460.

30 In addition to the entire A or B chains of a ricin-like toxin, it will be appreciated that the recombinant protein of the invention may contain only that portion of the A chain which is

necessary for exerting its cytotoxic effect. For example, the first 30 amino acids of the ricin A chain may be removed resulting in a truncated A chain which retains toxic activity. The truncated ricin or ricin-like A chain may be prepared by expression of a truncated gene or by

5 proteolytic degradation, for example with Nagarse (Funmatsu et al., *Jap. J. Med. Sci. Biol.* 23:264-267 (1970)). Similarly, the recombinant protein of the invention may contain only that portion of the B chain necessary for galactose recognition, cell binding and transport into the cell cytoplasm. Truncated B chains are described for example in E.P.

10 145,111. The A and B chains may be glycosylated or non-glycosylated. Glycosylated A and B chains may be obtained by expression in the appropriate host cell capable of glycosylation. Non-glycosylated chains may be obtained by expression in nonglycosylating host cells or by treatment to remove or destroy the carbohydrate moieties.

15 The proteins of the invention may be prepared using recombinant DNA methods. Accordingly, the nucleic acid molecules of the present invention may be incorporated in a known manner into an appropriate expression vector which ensures good expression of the protein. Possible expression vectors include but are not limited to

20 cosmids, plasmids, or modified viruses (e.g. replication defective retroviruses, adenoviruses and adeno-associated viruses), so long as the vector is compatible with the host cell used. The expression vectors are "suitable for transformation of a host cell", which means that the expression vectors contain a nucleic acid molecule of the invention and

25 regulatory sequences selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid molecule. Operatively linked is intended to mean that the nucleic acid is linked to regulatory sequences in a manner which allows expression of the nucleic acid.

30 The invention therefore contemplates a recombinant expression vector of the invention containing a nucleic acid molecule of the invention, or a fragment thereof, and the necessary regulatory

sequences for the transcription and translation of the inserted protein-sequence.

Suitable regulatory sequences may be derived from a variety of sources, including bacterial, fungal, viral, mammalian, or 5 insect genes (For example, see the regulatory sequences described in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). Selection of appropriate regulatory sequences is dependent on the host cell chosen as discussed below, and may be readily accomplished by one of ordinary skill in the 10 art. Examples of such regulatory sequences include: a transcriptional promoter and enhancer or RNA polymerase binding sequence, a ribosomal binding sequence, including a translation initiation signal. Additionally, depending on the host cell chosen and the vector employed, other sequences, such as an origin of replication, additional 15 DNA restriction sites, enhancers, and sequences conferring inducibility of transcription may be incorporated into the expression vector. It will also be appreciated that the necessary regulatory sequences may be supplied by the native A and B chains and/or its flanking regions.

The recombinant expression vectors of the invention 20 may also contain a selectable marker gene which facilitates the selection of host cells transformed or transfected with a recombinant molecule of the invention. Examples of selectable marker genes are genes encoding a protein such as G418 and hygromycin which confer resistance to certain drugs,  $\beta$ -galactosidase, chloramphenicol acetyltransferase, firefly 25 luciferase, or an immunoglobulin or portion thereof such as the Fc portion of an immunoglobulin preferably IgG. Transcription of the selectable marker gene is monitored by changes in the concentration of the selectable marker protein such as  $\beta$ -galactosidase, chloramphenicol acetyltransferase, or firefly luciferase. If the selectable marker gene 30 encodes a protein conferring antibiotic resistance such as neomycin resistance transformant cells can be selected with G418. Cells that have

- 50 -

incorporated the selectable marker gene will survive, while the other cells die. This makes it possible to visualize and assay for expression of recombinant expression vectors of the invention and in particular to determine the effect of a mutation on expression and phenotype. It will  
5 be appreciated that selectable markers can be introduced on a separate vector from the nucleic acid of interest.

The recombinant expression vectors may also contain genes which encode a fusion moiety which provides increased expression of the recombinant protein; increased solubility of the  
10 recombinant protein; and aid in the purification of the target recombinant protein by acting as a ligand in affinity purification. For example, a proteolytic cleavage site may be added to the target recombinant protein to allow separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein.  
15 Typical fusion expression vectors include pGEX (Amrad Corp., Melbourne, Australia), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the recombinant protein.

20 Recombinant expression vectors can be introduced into host cells to produce a transformant host cell. The term "transformant host cell" is intended to include prokaryotic and eukaryotic cells which have been transformed or transfected with a recombinant expression vector of the invention. The terms "transformed with", "transfected  
25 with", "transformation" and "transfection" are intended to encompass introduction of nucleic acid (e.g. a vector) into a cell by one of many possible techniques known in the art. Prokaryotic cells can be transformed with nucleic acid by, for example, electroporation or calcium-chloride mediated transformation. Nucleic acid can be  
30 introduced into mammalian cells via conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran mediated transfection, lipofectin, electroporation or microinjection.

Suitable methods for transforming and transfecting host cells can be found in Sambrook et al. (*Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other laboratory textbooks.

- 5            Suitable host cells include a wide variety of prokaryotic and eukaryotic host cells. For example, the proteins of the invention may be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus), yeast cells or mammalian cells. Other suitable host cells can be found in Goeddel, *Gene Expression Technology: Methods in*  
10 *Enzymology* 185, Academic Press, San Diego, CA (1991).

More particularly, bacterial host cells suitable for carrying out the present invention include *E. coli*, *B. subtilis*, *Salmonella typhimurium*, and various species within the genus' *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, as well as many other bacterial  
15 species well known to one of ordinary skill in the art. Suitable bacterial expression vectors preferably comprise a promoter which functions in the host cell, one or more selectable phenotypic markers, and a bacterial origin of replication. Representative promoters include the β-lactamase (penicillinase) and lactose promoter system (see Chang et al., *Nature* 20 275:615 (1978)), the trp promoter (Nichols and Yanofsky, *Meth in Enzymology* 101:155, (1983) and the tac promoter (Russell et al., *Gene* 20: 231, (1982)). Representative selectable markers include various antibiotic resistance markers such as the kanamycin or ampicillin resistance genes. Suitable expression vectors include but are not limited  
25 to bacteriophages such as lambda derivatives or plasmids such as pBR322 (Bolivar et al., *Gene* 2:9S, (1977)), the pUC plasmids pUC18, pUC19, pUC118, pUC119 (see Messing, *Meth in Enzymology* 101:20-77, 1983 and Vieira and Messing, *Gene* 19:259-268 (1982)), and pNH8A, pNH16a, pNH18a, and Bluescript M13 (Stratagene, La Jolla, Calif.).  
30 Typical fusion expression vectors which may be used are discussed above, e.g. pGEX (Amrad Corp., Melbourne, Australia), pMAL (New

- 52 -

England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ). Examples of inducible non-fusion expression vectors include pTrc (Amann et al., *Gene* 69:301-315 (1988)) and pET 11d (Studier et al., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, 5 San Diego, California, 60-89 (1990)).

Yeast and fungi host cells suitable for carrying out the present invention include, but are not limited to *Saccharomyces cerevisiae*, the genera *Pichia* or *Kluyveromyces* and various species of the genus *Aspergillus*. Examples of vectors for expression in yeast *S. cerevisiae* include pYEPSec1 (Baldari. et al., *Embo J.* 6:229-234 (1987)), pMFa (Kurjan and Herskowitz, *Cell* 30:933-943 (1982)), pJRY88 (Schultz et al., *Gene* 54:113-123 (1987)), and pYES2 (Invitrogen Corporation, San Diego, CA). Protocols for the transformation of yeast and fungi are well known to those of ordinary skill in the art.(see Hinnen et al., *Proc. Natl. Acad. Sci. USA* 75:1929 (1978); Itoh et al., *J. Bacteriology* 153:163 (1983), and Cullen et al. (*Bio/Technology* 5:369 (1987)).

Mammalian cells suitable for carrying out the present invention include, among others: COS (e.g., ATCC No. CRL 1650 or 1651), BHK (e.g. ATCC No. CRL 6281), CHO (ATCC No. CCL 61), HeLa (e.g., ATCC No. CCL 2), 293 (ATCC No. 1573) and NS-1 cells. Suitable expression vectors for directing expression in mammalian cells generally include a promoter (e.g., derived from viral material such as polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40), as well as other transcriptional and translational control sequences. Examples 20 of mammalian expression vectors include pCDM8 (Seed, B., *Nature* 329:840 (1987)) and pMT2PC (Kaufman et al., *EMBO J.* 6:187-195 (1987)).

Given the teachings provided herein, promoters, terminators, and methods for introducing expression vectors of an appropriate type into plant, avian, and insect cells may also be readily 30 accomplished. For example, within one embodiment, the proteins of the invention may be expressed from plant cells (see Sinkar et al., *J. Biosci* (Bangalore) 11:47-58 (1987), which reviews the use of

- 53 -

Agrobacterium rhizogenes vectors; see also Zambryski et al., Genetic Engineering, Principles and Methods, Hollaender and Setlow (eds.), Vol. VI, pp. 253-278, Plenum Press, New York (1984), which describes the use of expression vectors for plant cells, including, among others,  
5 pAS2022, pAS2023, and pAS2034).

Insect cells suitable for carrying out the present invention include cells and cell lines from *Bombyx*, *Trichoplusia* or *Spodotera* species. Baculovirus vectors available for expression of proteins in cultured insect cells (SF 9 cells) include the pAc series (Smith et al., *Mol.*  
10 *Cell Biol.* 3:2156-2165 (1983)) and the pVL series (Lucklow, V.A., and Summers, M.D., *Virology* 170:31-39 (1989)). Some baculovirus-insect cell expression systems suitable for expression of the recombinant proteins of the invention are described in PCT/US/02442.

Alternatively, the proteins of the invention may also be  
15 expressed in non-human transgenic animals such as, rats, rabbits, sheep and pigs (Hammer et al. *Nature* 315:680-683 (1985); Palmiter et al. *Science* 222:809-814 (1983); Brinster et al. *Proc. Natl. Acad. Sci. USA* 82:4438-4442 (1985); Palmiter and Brinster *Cell* 41:343-345 (1985) and U.S. Patent No. 4,736,866).

20 The proteins of the invention may also be prepared by chemical synthesis using techniques well known in the chemistry of proteins such as solid phase synthesis (Merrifield, *J. Am. Chem. Assoc.* 85:2149-2154 (1964)) or synthesis in homogenous solution (Houbenweyl, Methods of Organic Chemistry, ed. E. Wansch, Vol. 15 I and II, Thieme, Stuttgart (1987)).

The present invention also provides proteins comprising an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence linking the A and B chains, wherein the linker sequence contains a cleavage recognition site for a  
30 disease-specific protease. Such a protein could be prepared other than by recombinant means, for example by chemical synthesis or by conjugation of A and B chains and a linker sequence isolated and

purified from their natural plant, fungal or bacterial source. Such A and B chains could be prepared having the glycosylation pattern of the native ricin-like toxin.

N-terminal or C-terminal fusion proteins comprising the 5 protein of the invention conjugated with other molecules, such as proteins may be prepared by fusing, through recombinant techniques. The resultant fusion proteins contain a protein of the invention fused to the selected protein or marker protein as described herein. The recombinant protein of the invention may also be conjugated to other 10 proteins by known techniques. For example, the proteins may be coupled using heterobifunctional thiol-containing linkers as described in WO 90/10457, N-succinimidyl-3-(2-pyridyldithio-propionate) or N-succinimidyl-5 thioacetate. Examples of proteins which may be used to prepare fusion proteins or conjugates include cell binding proteins 15 such as immunoglobulins, hormones, growth factors, lectins, insulin, low density lipoprotein, glucagon, endorphins, transferrin, bombesin, asialoglycoprotein glutathione-S-transferase (GST), hemagglutinin (HA), and truncated myc.

#### **Utility of the Nucleic Acid Molecules and Proteins of the Invention**

The proteins of the invention may be used to specifically 20 inhibit or destroy mammalian cells affected by a disease or infection which have associated with such cells a specific protease, i.e., disease-specific, for example cancer cells or cells infected with a virus, fungus or parasite, all of which are encompassed within the term "disease-specific." 25 It is an advantage of the recombinant proteins of the invention that they have specificity for said cells without the need for a cell binding component. The ricin-like B chain of the recombinant proteins recognize galactose moieties on the cell surface and ensure that the protein is taken up by the diseased cell and released into the cytoplasm. 30 When the protein is internalized into a non-infected cell, cleavage of the heterologous linker would not occur in the absence of the disease-specific protease and the A chain will remain inactive bound to the B

- 55 -

chain. Conversely, when the protein is internalized into a diseased cell, the disease-specific protease will cleave the cleavage recognition site in the linker thereby releasing the toxic A chain.

- The specificity of a recombinant protein of the invention
- 5 may be tested by treating the protein with the disease-specific protease which is thought to be specific for the cleavage recognition site of the linker and assaying for cleavage products. Disease-specific proteases may be isolated from cancer cells or infected cells, or they may be prepared recombinantly, for example following the procedures in
- 10 Darket et al. (*J. Biol. Chem.* 254:2307-2312 (1988)). The cleavage products may be identified for example based on size, antigenicity or activity. The toxicity of the recombinant protein may be investigated by subjecting the cleavage products to an *in vitro* translation assay in cell lysates, for example using Brome Mosaic Virus mRNA as a template.
- 15 Toxicity of the cleavage products may be determined using a ribosomal inactivation assay (Westby et al., *Bioconjugate Chem.* 3:377-382 (1992)). The effect of the cleavage products on protein synthesis may be measured in standardized assays of *in vitro* translation utilizing partially defined cell free systems composed for example of a
- 20 reticulocyte lysate preparation as a source of ribosomes and various essential cofactors, such as mRNA template and amino acids. Use of radiolabelled amino acids in the mixture allows quantitation of incorporation of free amino acid precursors into trichloroacetic acid precipitable proteins. Rabbit reticulocyte lysates may be conveniently
- 25 used (O'Hare, *FEBS Lett.* 273:200-204 (1990)).

- The ability of the recombinant proteins of the invention to selectively inhibit or destroy animal cancer cells or cells infected with a virus or parasite may be readily tested *in vitro* using animal cancer cell lines or cell cultures infected with the virus or parasite of interest.
- 30 The selective inhibitory effect of the recombinant proteins of the invention may be determined, for example, by demonstrating the selective inhibition of viral antigen expression in infected mammalian

cells, the selective inhibition of general mRNA translation and protein synthesis in diseased cells, or selective inhibition of cellular proliferation in cancer cells or infected cells.

Toxicity may also be measured based on cell viability, for 5 example the viability of infected and non-infected cell cultures exposed to the recombinant protein may be compared. Cell viability may be assessed by known techniques, such as trypan blue exclusion assays.

In another example, a number of models may be used to test the cytotoxicity of recombinant proteins having a heterologous 10 linker sequence containing a cleavage recognition site for a cancer-associated matrix metalloprotease. Thompson, E.W. et al. (*Breast Cancer Res. Treatment* 31:357-370 (1994)) has described a model for the determination of invasiveness of human breast cancer cells *in vitro* by measuring tumour cell-mediated proteolysis of extracellular matrix and 15 tumour cell invasion of reconstituted basement membrane (collagen, laminin, fibronectin, Matrigel or gelatin). Other applicable cancer cell models include cultured ovarian adenocarcinoma cells (Young, T.N. et al. *Gynecol. Oncol.* 62:89-99 (1996); Moore, D.H. et al. *Gynecol. Oncol.* 65:78-82 (1997)), human follicular thyroid cancer cells (Demeure, M.J. et 20 al., *World J. Surg.* 16:770-776 (1992)), human melanoma (A-2058) and fibrosarcoma (HT-1080) cell lines (Mackay, A.R. et al. *Lab. Invest.* 70:781-783 (1994)), and lung squamous (HS-24) and adenocarcinoma (SB-3) cell lines (Spiess, E. et al. *J. Histochem. Cytochem.* 42:917-929 (1994)). An *in vivo* test system involving the implantation of tumours and 25 measurement of tumour growth and metastasis in athymic nude mice has also been described (Thompson, E.W. et al., *Breast Cancer Res. Treatment* 31:357-370 (1994); Shi, Y.E. et al., *Cancer Res.* 53:1409-1415 (1993)).

A further model may be used to test the cytotoxicity of 30 recombinant proteins having a heterologous linker sequence containing a cleavage recognition site for a cancer-associated Cathepsin

B protease is provided in human glioma (Mikkelsen, T. et al. *J. Neurosurgery*, 83:285-290 (1995)).

Similarly, the cytotoxicity of recombinant proteins having a heterologous linker sequence containing a cleavage recognition site  
5 for a malarial protease may be tested by a Plasmodium invasion assay using human erythrocytes infected with mature-stage merozoite parasites as described by McPherson, R.A. et al. (*Mol. Biochem. Parasitol.* 62:233-242 (1993)). Alternatively, in vitro cultures of human hepatic parenchymal cells may be used to evaluate schizont infectivity and  
10 Plasmodium merozoite generation.

With respect to models of viral infection and replication, suitable animal cells which can be cultured *in vitro* and which are capable of maintaining viral replication can be used as hosts. The toxicity of the recombinant protein for infected and non-infected  
15 cultures may then be compared. The ability of the recombinant protein of the invention to inhibit the expression of these viral antigens may be an important indicator of the ability of the protein to inhibit viral replication. Levels of these antigens may be measured in assays using labelled antibodies having specificity for the antigens. Inhibition of  
20 viral antigen expression has been correlated with inhibition of viral replication (U.S. Patent No. 4,869,903). Toxicity may also be assessed based on a decrease in protein synthesis in target cells, which may be measured by known techniques, such as incorporation of labelled amino acids, such as [3H] leucine (O'Hare et al., *FEBS Lett.* 273:200-204  
25 (1990)). Infected cells may also be pulsed with radiolabelled thymidine and incorporation of the radioactive label into cellular DNA may be taken as a measure of cellular proliferation. Toxicity may also be measured based on cell death or lysis, for example, the viability of infected and non-infected cell cultures exposed to the recombinant  
30 protein may be compared. Cell viability may be assessed by known techniques, such as trypan blue exclusion assays.

Although the primary specificity of the proteins of the invention for diseased cells is mediated by the specific cleavage of the cleavage recognition site of the linker, it will be appreciated that specific cell binding components may optionally be conjugated to the proteins 5 of the invention. Such cell binding components may be expressed as fusion proteins with the proteins of the invention or the cell binding component may be physically or chemically coupled to the protein component. Examples of suitable cell binding components include antibodies to cancer, viral or parasitic proteins.

10           Antibodies having specificity for a cell surface protein may be prepared by conventional methods. A mammal, (e.g. a mouse, hamster, or rabbit) can be immunized with an immunogenic form of the peptide which elicits an antibody response in the mammal. Techniques for conferring immunogenicity on a peptide include 15 conjugation to carriers or other techniques well known in the art. For example, the peptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassay procedures can be used with the immunogen as antigen 20 to assess the levels of antibodies. Following immunization, antisera can be obtained and, if desired, polyclonal antibodies isolated from the sera.

          To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from an immunized animal and 25 fused with myeloma cells by standard somatic cell fusion procedures thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art, (e.g. the hybridoma technique originally developed by Kohler and Milstein (*Nature* 256:495-497 (1975)) as well as other techniques such as the human B-cell hybridoma 30 technique (Kozbor et al., *Immunol. Today* 4:72 (1983)), the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., *Monoclonal Antibodies in Cancer Therapy* Allen R., Bliss,

Inc., pages 77-96 (1985)), and screening of combinatorial antibody libraries (Huse et al., *Science* 246:1275 (1989)). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with the peptide and the monoclonal antibodies can be isolated.

5       The term "antibody" as used herein is intended to include fragments thereof which also specifically react with a cell surface component. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above. For example, F(ab')2 fragments can be generated by  
10      treating antibody with pepsin. The resulting F(ab')2 fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

15      Chimeric antibody derivatives, i.e., antibody molecules that combine a non-human animal variable region and a human constant region are also contemplated within the scope of the invention. Chimeric antibody molecules can include, for example, the antigen binding domain from an antibody of a mouse, rat, or other species, with human constant regions. Conventional methods may be used to make chimeric antibodies containing the immunoglobulin variable region which recognizes a cell surface antigen (See, for  
20      example, Morrison et al., *Proc. Natl Acad. Sci. U.S.A.* 81:6851 (1985); Takeda et al., *Nature* 314:452 (1985), Cabilly et al., U.S. Patent No. 4,816,567; Boss et al., U.S. Patent No. 4,816,397; Tanaguchi et al., E.P. Patent No. 171,496; European Patent No. 173,494, United Kingdom Patent No. GB 2177096B). It is expected that chimeric antibodies would  
25      be less immunogenic in a human subject than the corresponding non-chimeric antibody.

30      Monoclonal or chimeric antibodies specifically reactive against cell surface components can be further humanized by producing human constant region chimeras, in which parts of the variable regions, particularly the conserved framework regions of the antigen-binding domain, are of human origin and only the hypervariable regions are of non-human origin. Such

- 60 -

immunoglobulin molecules may be made by techniques known in the art, (e.g. Teng et al., *Proc. Natl. Acad. Sci. U.S.A.*, 80:7308-7312 (1983); Kozbor et al., *Immunology Today* 4:7279 (1983); Olsson et al., *Meth. Enzymol.*, 92:3-16 (1982), and PCT Publication WO92/06193 or EP 5 239,400). Humanized antibodies can also be commercially produced (Scotgen Limited, 2 Holly Road, Twickenham, Middlesex, Great Britain.)

Specific antibodies, or antibody fragments, reactive against cell surface components may also be generated by screening expression libraries encoding immunoglobulin genes, or portions 10 thereof, expressed in bacteria with cell surface components. For example, complete Fab fragments, VH regions and FV regions can be expressed in bacteria using phage expression libraries (See for example Ward et al., *Nature* 341:544-546 (1989); Huse et al., *Science* 246:1275-1281 (1989); and McCafferty et al., *Nature* 348:552-554 (1990)). Alternatively, a 15 SCID-hu mouse, for example the model developed by Genpharm, can be used to produce antibodies, or fragments thereof.

The proteins of the invention may be formulated into pharmaceutical compositions for administration to subjects in a biologically compatible form suitable for administration *in vivo*. By 20 "biologically compatible form suitable for administration *in vivo*" is meant a form of the substance to be administered in which any toxic effects are outweighed by the therapeutic effects. The substances may be administered to living organisms including humans, and animals. Administration of a therapeutically active amount of the 25 pharmaceutical compositions of the present invention is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of a substance may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of antibody 30 to elicit a desired response in the individual. Dosage regime may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be

proportionally reduced as indicated by the exigencies of the therapeutic situation.

The nucleic acid molecules of the invention may be formulated into pharmaceutical compositions for administration to subjects in a biologically compatible form suitable for administration *in vivo*. By "biologically compatible form suitable for administration *in vivo*" is meant a form of the substance to be administered in which any toxic effects are outweighed by the therapeutic effects. The substances may be administered to living organisms including humans, and animals. Administration of a therapeutically active amount of the pharmaceutical compositions of the present invention is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of a substance may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of antibody to elicit a desired response in the individual. Dosage regime may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The active substance may be administered in a convenient manner such as by injection (subcutaneous, intravenous, intramuscular, etc.), oral administration, inhalation, transdermal administration (such as topical cream or ointment, etc.), or suppository applications. Depending on the route of administration, the active substance may be coated in a material to protect the compound from the action of enzymes, acids and other natural conditions which may inactivate the compound.

The compositions described herein can be prepared by *per se* known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with

a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985). On this basis, the compositions include, albeit not exclusively, 5 solutions of the substances in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

The pharmaceutical compositions may be used in 10 methods for treating animals, including mammals, preferably humans, with cancer or infected with a virus or a parasite. It is anticipated that the compositions will be particularly useful for treating patients with B-cell lymphoproliferative disease, (melanoma), mononucleosis, cytomegalic inclusion disease, malaria, herpes, shingles, hepatitis, 15 poliomyelitis, or infectious laryngotracheitis. The dosage and type of recombinant protein to be administered will depend on a variety of factors which may be readily monitored in human subjects. Such factors include the etiology and severity (grade and stage) of neoplasia, the stage of malarial infection (e.g. exoerythrocytic *vs.* erythrocytic), or 20 antigen levels associated with viral load in patient tissues or circulation.

As mentioned above, the novel recombinant toxic proteins and nucleic acid molecules of the present invention are useful in treating cancerous or infected cells wherein the cells contain a specific protease that can cleave the linker region of the recombinant toxic 25 protein. One skilled in the art can appreciate that many different recombinant toxic proteins can be prepared once a disease associated protease has been identified. For example, the novel recombinant toxic proteins and nucleic acid molecules of the invention may be used to treat CNS tumors. Muller et al. (1993) describe increased activity of 30 Insulin-type Growth Factor Binding Protein-3 (IGFBP-3) protease in the Cerebral Spinal Fluid of patients with CNS tumors. Cohen et al. (1992) claim that prostate-specific antigen (PSA) is an IGFBP-3 protease. The

- 63 -

pAP290 construct described above is a substrate for PSA. Conover et al. (1994) claim that cathepsin D is IGFBP-3 protease. The pAP276 described herein is a substrate for cathepsin D. Another example of a specific use of the invention is treatment of human glioma which has been shown 5 to produce cathepsin D (Mikkelsen, T. et al. *J. Neurosurge*, 83:285-290 (1995)). The pAP 214 and 272 define herein are substrates for cathepsin B.

In addition, the novel proteins and nucleic acid molecules of the present invention may be used to treat cystic fibrosis.

10 Hansen et al. (1995) describe how CF airway disease is characterized by neutrophil-dominated chronic inflammation with an excess of uninhibited neutrophil elastase (NE). NE levels in CF sputum are 350 times higher than that found in normal sputum. The pAP294 described herein is a substrate for neutrophil elastase.

15 As well, the novel proteins and nucleic acid molecules of the present invention may also be used to treat multiple sclerosis. Bever Jr. et al. (1994) implicate cathepsin B (possibly from inflammatory cells of hematogenous origin) in the demyelination found in multiple sclerosis. pAPs 214 and 272 defined herein present substrates for 20 cathepsin B.

The term "animal" as used herein includes all members of the animal kingdom including mammals, preferably humans.

The following non-limiting examples are illustrative of the present invention:

25 **EXAMPLES**

**Example 1**

**Cloning and Expression of Proricin Variants Activated by Disease-Specific Proteases**

**Isolation of total RNA**

30 The preproricin gene was cloned from new foliage of the castor bean plant. Total messenger RNA was isolated according to established procedures (Sambrook et al., *Molecular Cloning: A Lab*

- 64 -

*Manual* (Cold Spring Harbour Press, Cold Spring Harbour, (1989)) and cDNA generated using reverse transcriptase.

cDNA Synthesis:

Oligonucleotides, corresponding to the extreme 5' and 3' ends of the preproricin gene were synthesized and used to PCR amplify the gene. Using the cDNA sequence for preproricin (Lamb et al., Eur. J. Biochem., 145:266-270, 1985), several oligonucleotide primers were designed to flank the start and stop codons of the preproricin open reading frame. The oligonucleotides were synthesized using an Applied Biosystems Model 392 DNA/RNA Synthesizer. First strand cDNA synthesis was primed using the oligonucleotide Ricin1729C (Table 1). Three micrograms of total RNA was used as a template for oligo Ricin1729C primed synthesis of cDNA using Superscript II Reverse Transcriptase (BRL) following the manufacturer's protocol.

15 DNA Amplification and Cloning

The first strand cDNA synthesis reaction was used as template for DNA amplification by the polymerase chain reaction (PCR). The preproricin cDNA was amplified using the upstream primer Ricin-99 and the downstream primer Ricin1729C with Vent DNA polymerase (New England Biolabs) using standard procedures (Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, (Cold Spring Harbor Laboratory Press, 1989)). Amplification was carried out in a Biometra thermal cycler (TRIO-Thermalcycler) using the following cycling parameters: denaturation 95°C for 1 min., annealing 52°C for 1 min., and extension 72°C for 2 min., (33 cycles), followed by a final extension cycle at 72°C for 10 min. The 1846bp amplified product was fractionated on an agarose gel (Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, (Cold Spring Harbor Laboratory Press, 1989)), and the DNA purified from the gel slice using Qiaex resin (Qiagen) following the manufacturer's protocol. The purified PCR fragment encoding the preproricin cDNA was then ligated (Sambrook et al., Molecular Cloning: A Laboratory Manual, Second

- 65 -

Edition, (Cold Spring Harbor Laboratory Press, 1989)) into an Eco RV-digested pBluescript II SK plasmid (Stratagene), and used to transform competent XL1-Blue cells (Stratagene). Positive clones were confirmed by restriction digestion of purified plasmid DNA. Plasmid DNA was  
5 extracted using a Qiaprep Spin Plasmid Miniprep Kit (Qiagen).

DNA Sequencing

The cloned PCR product containing the putative preproricin gene was confirmed by DNA sequencing of the entire cDNA clone (pAP-144). Sequencing was performed using an Applied  
10 Biosystems 373A Automated DNA Sequencer, and confirmed by double-stranded dideoxy sequencing by the Sanger method using the Sequenase kit (USB). The oligonucleotide primers used for sequencing were as follows: Ricin267, Ricin486, Ricin725, Ricin937, Ricin1151, Ricini1399, Ricin1627, T3 primer  
15 (5'AATTAACCCCTCACTAAAGGG-3') (SEQ ID NO. 128) and T7 primer (5'GTAATACGACTCACTATAGGGC-3) (SEQ ID NO. 129). Sequence data was compiled and analyzed using PC Gene software package (intelligenetics). The sequences and location of oligonucleotide primers is shown in Table 1. The oligonucleotide primers shown in Table 1  
20 have been assigned the following sequence ID numbers:  
Ricin-109 is referred to herein as SEQ ID NO. 130;  
Ricin-99Eco is referred to herein as SEQ ID NO. 131;  
Ricin267 is referred to herein as SEQ ID NO. 132;  
Ricin486 is referred to herein as SEQ ID NO. 133;  
25 Ricin725 is referred to herein as SEQ ID NO. 134;  
Ricin 937 is referred to herein as SEQ ID NO. 135;  
Ricin 1151 is referred to herein as SEQ ID NO. 136;  
Ricin 1399 is referred to herein as SEQ ID NO. 137;  
Ricin 1627 is referred to herein as SEQ ID NO. 138;  
30 Ricin 1729C is referred to herein as SEQ ID NO. 139; and  
Ricin 1729C Xba is referred to herein as SEQ ID NO. 140.

Production and Cloning of Linker Variants

- 66 -

pAP144 cut with EcoRI was used as target for PCR pairs employing the Ricin109-Eco oligonucleotide (Ricin-109Eco primer: 5'-GGAGGAATCCGGAGATGAAACCGGGAGGAAATACTATTGTAAT-3' (SEQ ID No. 141)) and a mutagenic primer for the 5' half of the linker as well as the Ricin1729PstI primer (Ricin1729-PstI: 5'-GTAGGCGCTGCAGATAACTGCTGTCCTTCAG-3' (SEQ ID No. 142)) and a mutagenic primer for the 3' half of the linker. The cycling conditions used for the PCRs were 98 degrees C for 2 min.; 98C 1 min., 52C 1 min., 72C 1 min. 15 sec. (30 cycles); 72 degrees C 10min.; 4 degrees C soak. The PCR products were then digested by EcoRI and PstI respectively, electrophoresed on an agarose gel, and the bands purified by via glass wool spin columns. Triple ligations comprising the PCR product pairs (corresponding halves of the new linker) and pVL1393 vector digested with EcoRI and PstI were carried out. Recombinant clones were identified by restriction digests of plasmid miniprep DNA and the altered linkers confirmed by DNA sequencing. See Figure 45 as an example of the cloning strategy. Recombinant clones were identified by restriction digests of plasmid miniprep DNA and the altered linkers confirmed by DNA sequencing. Note that since all altered linker variants were cloned directly into the pVL1393 vector odd-numbered pAPs were no longer required or produced.

#### Isolation of Recombinant Baculoviruses

Insect cells *S. frugiperda* (Sf9), and *Trichoplusia ni* (Tn368 and BTI-TN-581-4 (High Five)) were maintained on EX-CELL 405 medium (JRH Biosciences) supplemented with 10% total calf serum (Summers et al., A Manual of Methods of Baculovirus Vectors and Insect Cell Culture Procedures, (Texas Agricultural Experiment Station, 1987)). Two micrograms of recombinant pVL1393 DNA was co-transfected with 0.5 microgram of BaculoGold AcNPV DNA (Pharmingen) into 2 x 10<sup>6</sup> Tn368 insect cells following the manufacturer's protocol (Gruenwald et al., Baculovirus Expression Vector System: Procedures and Methods Manual, 2nd Edition, (San

- 67 -

Diego, CA, 1993)). On day 5 post-transfection, media were centrifuged and the supernatants tested in limiting dilution assays with Tn368 cells (Summers et al., A Manual of Methods of Baculovirus Vectors and Insect Cell Culture Procedures, (Texas Agricultural Experiment Station, 5 1987)). Recombinant viruses in the supernatants were then amplified by infecting Tn368 cells at a multiplicity of infection (moi) of 0.1, followed by collection of day 3 to 5 supernatants. A total of three rounds of amplification were performed for each recombinant following established procedures (Summers et al., A Manual of Methods of 10 Baculovirus Vectors and Insect Cell Culture Procedures, (Texas Agricultural Experiment Station, 1987 and Gruenwald et al., Baculovirus Expression Vector System: Procedures and Methods Manual, 2nd Edition, (San Diego, CA, 1993)).

Expression of Mutant Proricin

15 Recombinant baculoviruses were used to infect 1X10<sup>7</sup> Tn368 or sf9 cells at an moi of 9 in EX-CELL 405 media (JRH Biosciences) with 25mM α-lactose in spinner flasks. Media supernatants containing mutant proricins were collected 3 or 4 days post-infection.

EXAMPLE 2

20 Harvesting and affinity column purification of pro-ricin variants

Protein samples were harvested three days post transfection. The cells were removed by centrifuging the media at 8288 g for ten minutes using a GS3 (Sorvall) centrifuge rotor. The supernatant was further clarified by centrifuging at 25400 g using a SLA-25 1500 rotor (Sorvall) for 45 minutes. Protease inhibitor phenylmethylsulfonyl fluoride (Sigma) was slowly added to a final concentration of 1mM. The samples were further prepared by adding lactose to a concentration of 20 mM (not including the previous lactose contained in the expression medium). The samples were concentrated 30 to 700 mL using a Prep/Scale-TFF Cartridge (2.5ft, 10K regenerated cellulose (Millipore)) and a Masterflex pump. The samples were then

- 68 -

dialysed for 2 days in 1X Column Buffer (50 mM Tris, 100 mM NaCl, 0.02% NaN<sub>3</sub>, pH 7.5) using dialysis tubing (10 K MWCO, 32 mm flat width(Spectra/Por)). Subsequently, the samples were clarified by centrifuging at 25400 g using a SLA-1500 rotor (Sorvall) for 45 minutes.

- 5        Following centrifugation, the samples were degassed and applied at 4 degrees C to a XK26/20 (Pharmacia) column (attached to a Pharmacia peristaltic pump, Pharmacia Single-path Monitor UV-1 Control and Optical Units, and Bromma LKB 2210 2-Channel Recorder) containing 20 mL of α-Lactose Agarose Resin (Sigma). The column was  
10      washed for 3 hours with 1X Column buffer. Elution of pro-ricin variant was performed by eluting with buffer (1X Column buffer (0.1% NaN<sub>3</sub>), 100 mM Lactose) until the baseline was again restored. The samples were concentrated using an Amicon 8050 concentrator (Amicon) with a YM10 76 mm membrane, utilizing argon gas to pressurize the chamber.  
15      The samples were further concentrated in Centricon 10 (Millipore) concentrators according to manufacturer's specifications.

**Purification of Variant pAP-Protein by gel filtration chromatography**

- In order to purify the pro-ricin variant from processed material produced during fermentation, the protein was applied to a  
20      SUPERDEX 75 (16/60) column and SUPERDEX 200 (16/60) column (Pharmacia) connected in series equilibrated with 50 mM Tris, 100mM NaCl, pH 7.5 containing 100 mM Lactose and 0.1% β-mercaptoethanol (βME). The flow rate of the column was 0.15 mL/min and fractions were collected every 25 minutes. The UV (280 nm) trace was used to  
25      determine the approximate location of the purified pAP-protein and thus determine the samples for Western analysis.

**Western analysis of column fractions**

- Fractions eluted from the SUPERDEX columns (Pharmacia) were analyzed for purity using standard Western blotting  
30      techniques. An aliquot of 10μL from each fraction was boiled in 1X sample buffer (62.6 mM Tris-C1, pH 6.8, 4.4% βME, 2% sodium dodecyl

- 69 -

sulfate (SDS), 5% glycerol (all from Sigma) and 0.002% bromophenol blue (Biorad)) for five minutes. Denatured samples were loaded on 12% Tris-Glycine Gels (Biorad) along with 50 ng of RCA<sub>60</sub> (Sigma) and 5 µL of kaleidoscope prestained standards (Biorad). Electrophoresis was  
5 carried out for ninety minutes at 100V in 25 mM Tris-Cl, pH 8.3, 0.1% SDS, and 192 mM glycine using the BioRad Mini Protean II cells (Biorad).

Following electrophoresis gels were equilibrated in transfer buffer (48 mM Tris, 39 mM glycine, 0.0375% SDS, and 20%  
10 Methanol) for a few minutes. PVDF Biorad membrane was presoaked for one minute in 100% methanol, rinsed in ddH<sub>2</sub>O and two minutes in transfer buffer. Whatman paper was soaked briefly in transfer buffer. Five pieces of Whatman paper, membrane, gel, and another five pieces of Whatman paper were arranged on the bottom cathode (anode) of the  
15 Pharmacia Novablot transfer apparatus (Pharmacia). Transfer was for one hour at constant current (2 mA/cm<sup>2</sup>).

Transfer was confirmed by checking for the appearance of the prestained standards on the membrane. Non-specific sites on the membrane were blocked by incubating the blot for thirty minutes in 1X  
20 Phosphate Buffered Saline (1X PBS; 137 mM NaCl, 2.7 mM KC1, 8 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4) with 5% skim milk powder (Carnation). Primary antibody (Rabbit α-ricin, Sigma) was diluted 1:3000 in 1X PBS containing 0.1% Tween 20 (Sigma) and 2.5% skim milk and incubated with blot for forty five minutes on a orbital shaker (VWR).  
25 Non-specifically bound primary antibody was removed by washing the blot for ten minutes with 1X PBS containing 0.2% Tween 20. This was repeated four times. Secondary antibody donkey anti-rabbit (Amersham) was incubated with the blot under the same conditions as the primary antibody. Excess secondary antibody was washed as  
30 described above. Blots were developed with the ECL Western Blotting detection reagents according to the manufacturer's instructions. Blots

- 70 -

were exposed to Medtec's Full Speed Blue Film (Medtee) or Amersham's ECL Hyperfilm (Amersham) for one second to five minutes. Film was developed in a KODAK Automatic Developer.

**Determination of lectin binding ability of pro-ricin variant**

5 An Immulon 2 plate (VDVR) was coated with 100 µl per well of 10µg/ml of asialofetuin and left overnight at 4°C. The plate was washed with 3X 300 µL per well with ddH<sub>2</sub>O using an automated plate washer (BioRad). The plate was blocked for one hour at 37°C by adding 300 µL per well of PBS containing 1% ovalbumin. The plate was  
10 washed again as above. Pro-ricin variant pAP-protein was added to the plate in various dilutions in 1X Baculo. A standard curve of RCA<sub>60</sub> (Sigma) from 1-10 ng was also included. The plate was incubated for 1 h at 37°C. The plate was washed as above. Anti-ricin monoclonal antibody (Sigma) was diluted 1:3000 in 1X PBS containing 0.5%  
15 ovalbumin and 0.1% tween-20, added at 100 µL per well and incubated for 1 h at 37°C. The plate was washed as above. Donkey-anti rabbit polyclonal antibody was diluted 1:3000 in 1X PBS containing 0.5% ovalbumin, 0.1% Tween-20, and added at 100µL per well and incubated for 1 h at 37°C. The plate was given a final wash as described above.  
20 Substrate was added to plate at 100µL per well (1 mg/ml o-phenylenediamine (Sigma), 1 µL/ml H<sub>2</sub>O<sub>2</sub>, 25 µL of stop solution (20% H<sub>2</sub>SO<sub>4</sub>) was added and the absorbance read (A490nm-A630nm) using a SPECTRA MAX 340 plate reader (Molecular Devices).

**Determination of pAP -Protein activity using the rabbit reticulocyte assay**

Ricin samples were prepared for reduction.

A)           RCA<sub>60</sub> = 3,500 ng/µL of RCA<sub>60</sub> + 997 µL 1xEndo buffer  
(25mM Tris, 25mM KCl, 5mM MGCl<sub>2</sub>, pH 7.6)

Reduction = 95 µL of 10ng/µL + 5 µL β-mercaptoethanol

- 71 -

B) Ricin variants

Reduction = 40 µL variant + 2 µL β-mercaptoethanol

The ricin standard and the variants were incubated for 30 minutes at room temperature.

5 **Ricin - Rabbit Reticulocyte lysate reaction**

The required number of 0.5 mL tubes were labelled. (2 tubes for each sample, + and - aniline). To each of the sample tubes 20 µL of 1X endo buffer was added, and 30 µL of buffer was added to the controls. To the sample tubes either 10 µL of 10ng/µL Ricin or 10µL of 10 variant was added. Finally, 30µL of rabbit reticulocyte lysate was added to all the tubes. The samples were incubated for 30 minutes at 30°C using the thermal block. Samples were removed from the eppendorf tube and contents added into a 1.5 mL tube containing 1 mL of TRIZOL (Gibco). Samples were incubated for 15 minutes at room temperature.

15 After the incubation, 200 µL of chloroform was added, and the sample was vortexed and spun at 12,000 g for 15 minutes at 4°C. The top aqueous layer from the samples was removed and contents added to a 1 mL tube containing 500 µL of isopropanol. Samples were incubated for 15 minutes at room temperature and then centrifuged at 12,000 for 15 20 minutes at 4°C. Supernatant was removed and the pellets were washed with 1 mL of 70% ethanol. Centrifugation at 12,000 g for 5 minutes at 4°C precipitated the RNA. All but approximately 20 µL of the supernatant was removed and air dried. Pellets from the other samples (+aniline samples) were dissolved in 20 µL of DEPC treated ddH<sub>2</sub>O. An 25 80 µL aliquot of 1 M aniline (distilled) with 2.8 M acetic acid was added to these RNA samples and transferred to a fresh 0.5 mL tube. The samples were incubated in the dark for 3 minutes at 60°C. RNA was precipitated by adding 100 µL of 95% ethanol and 5µL of 3M sodium acetate, pH 5.2 to each tube and centrifuging at 12,000 g for 30 minutes at

- 72 -

4°C. Pellets were washed with 1 mL 70% ethanol and centrifuged again at 12,000g for 5 minutes at 4°C to precipitate RNA. The supernatant was removed and air dried. These pellets were dissolved in 10µL of 0.1 X E buffer. To all samples, 10 µL of formamide loading dye was added. The  
5 RNA ladder (8 µL of ladder + 8 µL of loading dye) was also included. Samples were incubated for 2 minutes at 70°C on the thermal block. Electrophoresis was carried out on the samples using 1.2% agarose, 50% formamide gels in 0.1X E buffer + 0.2% SDS. The gel was run for 90 minutes at 75 watts. RNA was visualized by staining the gel in 1 µg/µL  
10 ethidium bromide in running buffer for 45 minutes. The gel was examined on a 302 nm UV box, photographed using the gel documentation system and saved to a computer disk.

**Results:**

**Protein Expression Yields**

15 Aliquots were taken at each stop of the harvesting/purification and tested. Yields of functional ricin variant were determined by ELISA. Typical results of an 2400 mL prep of infected *T. ni* cells are given below.

<u>Aliquot</u>	<u>µg pAP 220</u>
20 Before concentration and dialysis	6000
After concentration and dialysis	4931
alpha- Lactose agarose column flow through	219
alpha- Lactose agarose column elution	1058

25 Yield: 1058/6000 = 17.6%

**Purification of pAP -Protein and Western Analysis of column fractions**

Partially purified pAP-protein was applied to Superdex 75 and 200 (16/60) columns connected in series in order to remove the

contaminating non-specifically processed pAP-protein. Eluted fractions were tested via Western analysis as described above and the fractions containing the most pure protein were pooled, concentrated and re-applied to the column. The variant was applied a total of three times to 5 the column. Final purified pAP-protein has less than 1% processed variant.

The purified pAP-protein was tested for susceptibility to cleavage by the particular protease and for activation of the A-chain of the proricin variant, (inhibition of protein synthesis). Typically, pAP-protein 10 was incubated with and without protease for a specified time period and then electrophoresed and blotted. Cleaved pAP will run as two 30 kDa proteins (B is slightly larger) under reducing (SDS-PAGE) conditions. Unprocessed pAP-protein, which contains the linker region, will run at 60 kDa.

15 **Activation of pAP -Protein variant with Specific Protease**

Activation of protease treated pAP-protein is based on the method of *May et al.* (EMBO Journal. 8 301-8, 1989). Activation of ricin A chain upon cleavage of the intermediary linker results in catalytic depurination of the adenosine 4325 residue of 28S or 26S rRNA. This 20 depurination renders the molecule susceptible to amine-catalyzed hydrolysis by aniline of the phosphodiester bond on either side of the modification site. The result is a diagnostic 390 base band. As such, reticulocyte ribosomes incubated with biochemically purified ricin A chain, released the characteristic RNA fragment upon aniline treatment 25 of isolated rRNA (May, M.J. et al. Embo. Journal, 8:301-308 at 302-303 (1989)). It is on this basis that the assay allows for the determination of activity of a ricin A chain which has been cleaved from the intact unit containing a particular variant linker sequence.

**EXAMPLE 3**

30 **In Vitro Protease Digestion of Proricin Variants:**

Affinity-purified proricin variant is treated with individual disease-specific proteases to confirm specific cleavage in the linker

region. Ricin-like toxin variants are eluted from the lactose-agarose matrix in protease digestion buffer (50mM NaCl, 50mM Na-acetate, pH 5.5, 1mM dithiothreitol) containing 100mM lactose. Proricin substrate is then incubated at 37°C for 60 minutes with a disease-specific protease.

- 5 The cleavage products consisting ricin A and B chains are identified using SDS/PAGE (Sambrook et al., Molecular Cloning: a Laboratory Manual, 2nd. ed., Cold Spring Harbor Press, 1989), followed by Western blot analysis using anti-ricin antibodies (Sigma).

Cathepsin B may be obtained from Medcor or Calbiochem.

- 10 Matrix metalloproteinases may be prepared substantially as described by Lark, M.W. et al. (*Proceedings of the 4th International Conference of the Imflammation Research Association Abstract* 145 (1988)) and Welch, A.R. et al. (*Arch. Biochem. Biophys.* 324:59-64 (1995)). Candida acid protease may be prepared substantially as described in Remold, H.H. et  
15 al. (*Biochim. Biophys. Acta* 167:399-406 (1968)), Ray, T.L. and Payne, C.D. (*Infect. Immunol.* 58:508-514 (1990)) and Fusek, M. et al. (*FEBS Lett.* 327:108-112 (1993)). Hepatitis A protease may be prepared as described in Jewell, D.A. et al. (*Biochemistry* 31:7862-7869 (1992)). Plasmodium proteases may be prepared as described in Goldberg, D.E. et al. (*J. Exp. 20 Med.* 173:961-969 (1991)) and Cooper, J.A. and Bujard, H. (*Mol. Biochem. Parasitol.* 56:151-160 (1992)).

In Vitro Cytotoxicity Assay:

- Human ovarian cancer cells (e.g. MA148) are seeded in 96-well flat-bottom plates and are exposed to ricin-like toxin variants or control  
25 medium at 37°C for 16 h. The viability of the cancer cells is determined by measuring [<sup>35</sup>S]methionine incorporation and is significantly lower in wells treated with the toxin variants than those with control medium.

In Vivo Tumour Growth Inhibition Assay:

- 30 Human breast cancer (e.g. MCF-7) cells are maintained in suitable medium containing 10% fetal calf serum. The cells are grown, harvested and subsequently injected subcutaneously into

ovariectomized athymic nude mice. Tumour size is determined at intervals by measuring two right-angle measurements using calipers. In animals that received ricin-like toxin variants containing the matrix metalloproteinase-sensitive linkers, tumour size and the rate of  
5 tumour growth are lower than animals in the control group.

In Vivo Tumour Metastasis Assay:

The metastasis study is performed substantially as described in Honn, K.V. et al. (*Biochem. Pharmacol.* 34:235-241 (1985)). Viable B16a melanoma tumour cells are prepared and injected subcutaneously into  
10 the left axillary region of syngeneic mice. The extent of tumour metastasis is measured after 4 weeks. The lungs are removed from the animals and are fixed in Bouin's solution and macroscopic pulmonary metastases are counted using a dissecting microscope. In general without therapeutic intervention, injection of  $10^5$  viable tumour cells  
15 forms approximately 40-50 pulmonary metastases. The number of metastases in animal treated with proricin variants containing cathepsin B-sensitive linkers is substantially lower.

EXAMPLE 4

In Vitro Protease Digestion of Proricin Variants by Cancer Proteases

20 Cathepsin B or MMP-9

The general protocol for proricin digestion by cancer proteases is described in Examples 2 and 3.

In Vitro Protease Digestion of Cathepsin B Proricin Variant

Affinity-purified mutant proricin is treated with individual  
25 disease-specific proteases to confirm specific cleavage in the linker region. The proricin substrate is digested in a Cathepsin B protease buffer (50 mM Sodium acetate, 2 mM EDTA, 0.05% Triton) at 40°C. Two hours and overnight (16 hr) digestion reactions are carried out using 100ng of proricin substrate and 100 and 618 ng of Cathepsin B protease  
30 per reaction (CALBIOCHEM, USA). The cleavage products of proricin (ricin A and B chains) are identified using SDS/PAGE (Sambrook et al., Molecular cloning: a laboratory Manual, 2nd. ed., Cold Spring Harbor

- 76 -

Press, 1989), followed by Western blot analysis using anti-ricin antibodies (Sigma).

In Vitro Protease Digestion of MMP-9 Proricin Variant

Affinity-purified mutant proricin is treated with individual disease-specific proteases to confirm specific cleavage in the linker region. The proricin substrate is digested in 1X column buffer (100 mM NaCl, 50 mM Tris, PH 7.5) at 37°C. Two hours and overnight (16 hr) digestion reactions are set up using 50 ng of MMP-9 proricin substrate and 20 and 200 ng of MMP-9 protease per reaction (CALBIOCHEM, USA). The cleavage products of proricin (ricin A and B chains) are identified using SDS/PAGE (Sambrook et al., Molecular cloning: a laboratory Manual, 2nd. ed., Cold Spring Harbor Press, 1989), followed by Western blot analysis using anti-ricin antibodies (Sigma).

The protocol for Western analysis of ricin chains is described in Example 2.

Results

Figures 48 and 49 illustrate Western blots showing the cleavage of the protease-sensitive linkers by cathepsin B (pAP 214) and MMP-9 (pAP 220) respectively. Without protease digestion, the proricin variant appears as a single band at approximately 60 kDa (Lane B of Figure 48 and Lane A of Figure 49). Wild type ricin A chain and B chain appear as two disparate bands at approximately 30 kDa (Lane A of Figure 48 and Lane E of Figure 49). Increasing extent of proricin cleavage can clearly be observed with increasing protease concentration (Lanes C and D of Figure 48 and Lanes B-C of Figure 49).

EXAMPLE 5

In vitro protease digestion of various proricin variants by their corresponding proteases.

The general protocol for proricin digestion by coresponding proteases was as desribed in Examples 2 and 3 and should be considered in connection with the digestions described below.

**Cleavage of pAP-222 protein with the Matrix Metalloproteinase 2 (MMP-2)**

Affinity-purified mutant proricin is treated with individual disease-specific proteases to confirm specific cleavage in the linker  
5 region.

The pAP-222 protein sample (1.0 ug) was digested with the MMP-2 protease (1.0 ug) overnight at 37° C. The total volume of the digestion reaction was 21.5 ul, and 0.250 ug of the reaction sample was loaded on a protein gel. The MMP-2 protease was purchased from  
10 Calbiochem-Novabiochem Corporation, USA.

**Cleavage of pAP-248 protein with the Human Cytomegalovirus (HCMV) protease**

Affinity-purified mutant proricin is treated with individual disease-specific proteases to confirm specific cleavage in the linker  
15 region.

The pAP-248 protein sample (1.19 ug) was digested with the HCMV protease (1.13 ug) overnight at 37°C. The total volume of the digestion was 10.5 ul, and 0.279 ug of the reaction sample was loaded on a protein gel. The HCMV was purchased from BACHEM Bioscience Inc., USA.

20 **Cleavage of pAP-256 protein with the Hepatitis A virus 3C (HAV 3C) protease**

Affinity-purified mutant proricin is treated with individual disease-specific proteases to confirm specific cleavage in the linker region.

25 The pAP-256 protein sample (1.26 ug) was digested with the HAV 3C protease (5 ug) overnight at 37°C. The total volume of the digestion was 12.5 ul, and 0.302 ug of the digestion sample was loaded on a protein gel. The HAV 3C protease was a gift from Dr. G. Lawson from Bates Collage, Main, USA.

30 **Cleavage of pAP-270 protein with the Matrix Metalloproteinase 2 (MMP-2)**

- 78 -

Affinity-purified mutant proricin is treated with individual disease-specific proteases to confirm specific cleavage in the linker region.

The pAP-270 protein sample (0.120 ug) was digested with the MMP-2  
5 protease (0.25 ug) overnight at 37° C. The total volume of the digestion reaction was 22.5 ul, and 0.106 ug of the reaction sample was loaded on a protein gel. The MMP-2 protease was purchased from Calbiochem-Novabiochem Corporation, USA.

**Cleavage of pAP-288 protein with tPA plasminogen tissue activator**

10 Affinity-purified mutant proricin is treated with individual disease-specific proteases to confirm specific cleavage in the linker region. The pAP-288 protein sample (1.65 ug) was digested with the t-PA protease (0.5 ug) overnight at 37° C. The total volume of the digestion reaction was 55 ul, and 0.6 ug of the reaction sample was  
15 loaded on a protein gel. The t-PA was purchased from Sigma Chemical Co., USA.

**Cleavage of pAP-294 protein with human neutrophil elastase**

20 Affinity-purified mutant proricin is treated with individual disease-specific proteases to confirm specific cleavage in the linker region.

The pAP-256 protein sample (0.6 ug) was digested with the Elastase protease (5 ug) at 25° C for one hour. The total volume of the digestion reaction was 52.5 ul, and 0.171 ug of the digestion sample was loaded on a protein gel. The Human Neutrophil Elastase protease was purchased  
25 from Cedarlane Laboratories Limited, Canada.

**Cleavage of pAP-296 protein with calpain**

Affinity-purified mutant proricin is treated with individual disease-specific proteases to confirm specific cleavage in the linker region. The pAP-296 protein sample (2.05 ug) was digested with the  
30 Calpain protease (10 ug) overnight at 37° C. The total volume of the digestion reaction was 35 ul and 0.761 ug of the reaction sample was

loaded on a protein gel. The Calpain protease was purchased from Sigma Chemical Co., USA

### Results

Figures 52, 54, 58 & 66(MMP-2), 60, 64 and 62 show the cleavage 5 of proteases of linkers by HCMV, HAV 3C, MMP-2, t-PA, calpain, and human neutrophil elastase respectively. Without protease digestion, the proricin variants appear as a single band at approximately 60kDA (Lane A in connection with Figure 52; Lane B of Figure 54; Lane A of Figure 58; Lane B of Figure 60; and Lane C of Figure 62; lane B of Figure 10 64 and lane B of Figure 66). Wild type ricin chain A and B appear as two bands at approximately 30kDA (see for example Lanes C and D of Figure 52) proricin cleavage can clearly be observed with the appearance of 30kDA bands in connection with the protein which has been digested by the respective protease (see Lane B of Figure 52; Lane C of Figure 54; or 15 Lane B of Figure 58 for examples).

### EXAMPLE 6

#### In Vitro Translation Assay (Activation by Cancer Proteases Cathepsin B or MMP-9)

The general protocol for the rabbit retoculocyte lysate reaction to 20 test the cytotoxicity of cancer protease-activated proricin is described briefly in Example 3 and is described in more detail in Example 2.

### Results

Activation of pAP 214 and pAP 220 proricin variants by cathepsin B and MMP-9, based on the method of May et al. (EMBO J. 25 8:301-308, 1989), is illustrated in Figures 50 and 51 respectively. The appearance of the 390 base pair product (positive control) is observed in Lane F of Figure 50 and Lane G of Figure 51. This 390 base pair product is absent in the negative control lanes. Without cathepsin or MMP-9 activation, no or minimal N-glycosidase activity in the pAP 214 variant 30 (Lanes H to L, Figure 50) or the pAP 220 variant (Lanes A to E, Figure 51) was observed. When the pAP 214 variant and the pAP 220 variant were activated by cathepsin or MMP-9 respectively, appearance of the 390 base

- 80 -

pair product was observed in a proricin concentration-dependent manner (Lanes A to E of Figure 50 and Lanes H to L of Figure 51). The present experimental series demonstrated the successful and selective activation of proricin variants by cancer-associated proteases.

5 **EXAMPLE 7**

The general protocol for the rabbit reticulocyte lysate reaction is described briefly in Example 3 and is described in more detail in Example 2, all of which compliments the description below.

10 **Depurination of Rabbit Reticulocyte 28S Ribosomal RNA by Digested and Undigested Ricin Variants**

Affinity-purified mutant proricin mutants which were previously digested with the disease-specific protease, were reduced with 5% 2-mercaptoethanol then diluted to 100ng, 14.2ng, 2.0ng, 291pg, and 41.7pg with 1 X ENDO buffer(25mM Tris pH 7.6, 25mM KCl, 5mM 15 MgCl<sub>2</sub>) and incubated with rabbit reticulocyte lysate, untreated (Promega) for 30minutes at 30(C. To compare the digested with the undigested proricin variant, the proricin in digestion buffer (according to the specific digestion protocol) was treated in the same manner as the digested sample. As a positive and negative control, 10ng of ricin A 20 chain and 1 X ENDO buffer consecutively, was incubated with rabbit reticulocyte lysate, untreated, for 30 min at 30°C.

**Aniline Cleavage of rRNA and Gel Fractionation**

Total RNA was then extracted from reticulocyte lysate translation mixtures with Trizol reagent (Gibco-BRL) as per 25 manufacturer's instructions. The RNA was incubated with 80ul of 1M aniline (distilled) with 2.8M acetic acid for 3 min at 60(C in the dark. Ethanol-precipitated RNA samples were dissolved in 20ul of 50% formamide, 0.1X E buffer (3.6mM Tris, 3mM NaH<sub>2</sub>PO<sub>4</sub>, 0.2mM EDTA), and 0.05% xylene cyanol. 10ul of this was heated to 70(C for 2 minutes, 30 loaded and electrophoresed in 1.2% agarose, 0.1X E buffer, and 50% formamide gel with RNA running buffer (0.1 X E buffer, 0.2% SDS).

**Results**

Activation of pAP-248 proricin variant by HCMV; pAP-256 by HAV3C protease; pAP-270 by MMP-2 protease; pAP-288 by t-PA protease; pAP-294 by human neutrophil elastase; pAP-296 by calpain; and pAP-222 by MMP-2 is illustrated in Figures 52, 55, 59, 61, 63, 65, and 67 respectively. The appearance of the 390 base pair product (deposit of control) is observed in lane L of Figures 53, 55, 61, 63, 65 and 67. The 390 base pair product is observed in lane A of Figures 59 (activation of pAP-270 by MMP-2). This 390 base pair product is absent in the negative control lanes. Without the specific protease activation, no or minimal activity is seen in the lanes which contained only the proricin variant without digestion (see lane A, B, C, D, and E of Figures 53, 55, 61, 63, 65, and 67). The same observation is made in connection with pAP-270 in Figure 59, however, the undigested lanes appear as H, I, J, K and L. When the variant was activated by its respective protease, there is an appearance of the 390 base pair product in a proricin concentration-dependent manner (see Lanes H, I, J, K and L of Figure 53, 55, 61, 63, 65, and 67 and Lanes A, B, C, D, and E of Figure 59). The present experimental series demonstrate the successful and selective activation of the identified proricin variants by selective corresponding proteases.

20 **EXAMPLE 8**

**Procedure for Examining the Cytotoxicity of Ricin and Ricin Variants on the COS-1 Cell Line**

**Cell Preparation**

After washing with 1XPBS (0.137 M NaCl, 2.68 mM KCl, 8.10 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.47 mM KH<sub>2</sub>PO<sub>4</sub>), cells in log phase growth were removed from plates with 1X trypsin/EDTA (Gibco/BRL). The cells were centrifuged at 1100 rpm for 3 min, resuspended in Dulbecco's Modified Eagle Medium containing 10%FBS and 1X pen/strep, and then counted using a haemocytometer. They were adjusted to a concentration of 5 X 10<sup>4</sup> cells•ml<sup>-1</sup>. One hundred microliters per well of cells was added to wells 2B - 2G through to wells 9B - 9G of a Falcon 96 well tissue culture plate. A separate 96 well tissue culture plate was

- 82 -

used for each sample of Ricin or Ricin variant. The plates were incubated at 37(C with 5% CO<sub>2</sub> for 24 hours.

### Toxin Preparation

The Ricin and Ricin variants were sterile filtered using a 0.22μm filter (Millipore). The concentration of the sterile samples were then quantified by A<sub>280</sub> and confirmed by BCA measurements (Pierce). For the variants digested with the protease in vitro, the digests were carried out as described in the digestion procedure for each protease. The digests were then diluted in the 1000 ng•ml<sup>-1</sup> dilution and sterile filtered. The Ricin and the undigested pAP214 in the pAP 214 cytotoxicity data were treated in the same manner but without the Cathepsin B treatment. Ricin and Ricin variants were serially diluted to the following concentrations: 1000 ng•ml<sup>-1</sup>, 100 ng•ml<sup>-1</sup>, 10 ng•ml<sup>-1</sup>, 1 ng•ml<sup>-1</sup>, 0.1 ng•ml<sup>-1</sup>, 0.01 ng•ml<sup>-1</sup>, 0.001 ng•ml<sup>-1</sup> with media containing 10%FBS and 1X pen/strep.

### Application of Toxin or Variants to Plates

Columns 2 to 9 were labeled: control, 1000 ng•ml<sup>-1</sup>, 100 ng•ml<sup>-1</sup>, 10 ng•ml<sup>-1</sup>, 1 ng•ml<sup>-1</sup>, 0.1 ng•ml<sup>-1</sup>, 0.01 ng•ml<sup>-1</sup>, 0.001 ng•ml<sup>-1</sup> consecutively. The media was removed from all the sample wells with a multichannel pipettor. For each plate of variant and toxin, 50μl of media was added to wells 2B to 2G as the control, and 50μl of each sample dilution was added to the corresponding columns. For the pAP220 + MMP-9 data, the plates were incubated for one hour at 37(C with 5% CO<sub>2</sub>, then washed once and replaced with media, then incubated for 48 hours at 37(C with 5% CO<sub>2</sub>. For the pAP 214 + Cathepsin B data, the toxin was left on the plates and incubated for 24 hours at 37(C with 5% CO<sub>2</sub>, then 50 μl of media was added to the wells with the toxin and incubated for another 24 hours at 37(C with 5% CO<sub>2</sub>.

### Sample Application

- 83 -

The whole amount of media (and/or toxin)was removed from each well with a multichannel pipettor, and replaced with 100  $\mu$ l of the substrate mixture (Promega Cell Titer 96 Aqueous Non-Radioactive Cell Proliferation Assay Kit). The plates were incubated at 37°C with 5% CO<sub>2</sub> 5 for 2 to 4 hours, and subsequently read with a Spectramax 340 96 well plate reader at 490nm. The IC<sub>50</sub> values were calculated using the GRAFIT software program.

### Results

In experiments with pAP-214 and Cathepsin B incubated with 10 COS-1 cells, it may be seen that cells incubated with pAP-214 alone, pAP-214 was ineffective at causing cell death (see Figure 56). However, the cytotoxicity of pAP-214 digested with Cathepsin B behaves similarly to the ricin control in COS-1 cells. This is also illustrated in Figure 56. Similarly, the cytotoxicity of undigested pAP-220 when incubated with 15 COS-1 cells is lower than the cytotoxicity observed with COS-1 cells incubated with pAP-220 digested with MMP-9. Indeed the results suggest that the toxicity of digested pAP-220 is greater than that of ricin. (See Figure 57).

### EXAMPLE 9

20 **Procedure for Examining the Cytotoxicity of Ricin and Ricin Variants on Various Tissue Culture Cell Lines**

#### Cell Preparation

After washing with 1XPBS (1.37M NaCl, 26.8mM KCl, 81mM Na<sub>2</sub>HPO<sub>4</sub>, 14.7mM KH<sub>2</sub>PO<sub>4</sub> ), cells in log phase growth were removed 25 from plates with 1X trypsin/EDTA (Gibco/BRL). The cells were centrifuged at 1100 rpm for 3 min, resuspended in media containing 10%FBS and 1X pen/strep (media used depended on the cell line being tested), and then counted using a haemocytometer. They were adjusted to a concentration of 5 X 10<sup>4</sup> cells•ml<sup>-1</sup> (faster growing cell lines were 30 adjusted to 2 X10<sup>4</sup> cells•ml<sup>-1</sup>). One hundred microliters per well of cells was added to wells 2B - 2G through to wells 9B - 9G of a Falcon 96 well

- 84 -

tissue culture plate. A separate 96 well tissue culture plate was used for each sample of Ricin or Ricin variant. The plates were incubated at 37(C with 5% CO<sub>2</sub> for 24 hours.

### Toxin Preparation

5       The Ricin and Ricin variants were sterile filtered using a 0.22μm filter (Millipore). The concentration of the sterile samples were then quantified by A<sub>280</sub> and confirmed by a BCA measurement (Pierce). Ricin and Ricin variants were serially diluted to the following concentrations:  
10 3000 ng•ml<sup>-1</sup>, 300 ng•ml<sup>-1</sup>, 30 ng•ml<sup>-1</sup>, 3 ng•ml<sup>-1</sup>, 0.3 ng•ml<sup>-1</sup>, 0.03ng•ml<sup>-1</sup>, 0.003 ng•ml<sup>-1</sup> with media containing 10%FBS and 1X pen/strep.

### Application of Toxin or Variants to Plates

Columns 2 to 9 were labeled: control, 0.001 ng•ml<sup>-1</sup>, 0.01 ng•ml<sup>-1</sup>, 0.1 ng•ml<sup>-1</sup>, 1ng•ml<sup>-1</sup>, 10 ng•ml<sup>-1</sup>, 100 ng•ml<sup>-1</sup>, 1000 ng•ml<sup>-1</sup> consecutively. For each plate of variant and toxin, 50μl of media was added to wells 2B to 2G as the control, and 50μl of each sample dilution was added to the corresponding columns containing 100μl per well of cells (i.e. 50 μl of the 3000 ng•ml<sup>-1</sup> dilution added to the wells B-G in column 9, labeled 1000 ng•ml<sup>-1</sup>). The plates were incubated for 48 hours at 37(C with 5% CO<sub>2</sub>.

### Sample Application

An amount of 140μl was removed from each well with a multichannel pipettor, and replaced with 100 μl of the substrate mixture (Promega Cell Titer 96 Aqueous Non-Radioactive Cell Proliferation Assay Kit). The plates were incubated at 37(C with 5% CO<sub>2</sub> for 2 to 4 hours, and subsequently read with a Spectramax 340 96 well plate reader at 490nm. The IC<sub>50</sub> values were calculated using the GRAFIT software program.

### Results

Referring to Table 2, it may be seen that the survival of cells is correlated with the proricin variant and the cell specific protease produced by the cell type. For example, in the HT1080 cell line, both pAP-214 and pAP-220 required only 2-1/2 times the amount of ricin to 5 achieve the same level of cytotoxicity. On the other hand, pAP-224 required 193 times the amount of ricin to achieve the same level of cell death. As well, it may be seen that in the cells where expression of Cathepsin D is found, pAP-214 and 220 were more effective at causing cell death than ricin and more effective than pAP-224. Details 10 concerning the various cells types used in these experiments are outlined below.

**COS-1 (African Green Monkey Kidney Cells)**

This is an SV40 transformed cell line which was prepared from established simian cells CV-1. (Reference: Gluzman, Y. (1975) Cell, 23, 15 175 - 182)(ATCC CRL 1650)

**HT-1080 Human Fibrosarcoma**

(ATCC CCL 121) This cell line was shown to produce active MMP-9 in tissue culture. References: Moore et al. (1997) Gynecologic Oncology 65, 83-88.

20 **9L Rat Glioblastoma**

Glioblastomas are generally associated with cathepsin B expression. Levels of cathepsin B expression correspond to the extent of progression of malignancy i.e. highest levels for glioblastomas over anaplastic astrocytomas over low-grade gliomas and normal brain tissue. The 9L 25 cell line was provided by Dr. William Jia of the B.C. Cancer Agency.

References: Mikkelsen et al. (Aug. 1995) Journal of Neurosurgery 83(2), 285-290. Nakano et al. (1995) J. of Neurosurgery 83(2), 298-307.

**MCF-7 Human Breast Cancer Cell Line (Epithelial)**

(ATCC CRL 1555) In the absence of estrogen cathepsin B has not been 30 shown to be elevated relative to normal cells. It can be induced with estrogen to produce Cathepsin D. Production of MMP-9 is unknown.

- 86 -

Having illustrated and described the principles of the invention in a preferred embodiment, it should be appreciated by those skilled in the art that the invention can be modified in arrangement and detail without departure from such principles. We claim all modifications  
5 coming within the scope of the following claims.

All publications, patents and patent applications referred to herein are incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in  
10 its entirety.

**FULL CITATIONS FOR CERTAIN REFERENCES REFERRED TO IN  
THE SPECIFICATION**

- Bever Jr., C.T., Panitch, H.S., and Johnson, K.P. (1994) Neurology 44(4), 745-8. Increased cathepsin B activity in peripheral blood mononuclear 5 cells of multiple sclerosis patients.
- Cohen, P., Graves, H.C., Peehl, D.M., Kamarei, M., Giudice, L.C., and Rosenfeld, R.G. (1992) Journal of Clinical Endocrinology and Metabolism 75(4), 1046-53. Prostate-specific antigen (PSA) is an insulin-like growth factor binding protein-3 protease found in seminal plasma.
- 10 Conover, C.A. and De Leon, D.D. (1994) J. Biol. Chem. 269(10), 7076-80. Acid activated insulin-like growth factor-binding protein-3 proteolysis in normal and transformed cells. Role of cathepsin D.
- Hansen, G., Schuster, A., Zubrod, C., and Wahn, V. (1995) Respiration 62(3), 117-24. Alpha 1-proteinase inhibitor abrogates proteolytic and 15 secretagogue activity of cystic fibrosis sputum.
- Muller, H.L., Oh, Y., Gargosky, S.E., Lehrnbecher, T., Hintz, R.L., and Rosenfeld, R.G. (1993) Journal of Clinical Endocrinology and Metabolism 77(5), 1113-9. Concentrations of insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3), IGF, and IGFBP-3 protease activity in 20 cerebrospinal fluid of children with leukemia, central nervous system tumor, or meningitis.

TABLE 1

**Table I - Sequence and Location of Oligonucleotide Primers**

Name of Primer	Primer Sequence †	Corresponds to preproricin nucleotide numbers: (see Figures 8-10)
Ricin-109	5' - GGAGATGAAACCGGGAGGAAATACTATTGTAAT-3'	27 to 59
Ricin-99Eco	5' - <u>GCGGAATTCCGGGAGGAAATACTATTGTAAT</u> -3'	37 to 59
Ricin267	5' - ACGGTTTATTAGTTAGTTGA-3'	300 to 317
Ricin486	5' - ACTTGCTGGTAATCTGAG -3'	519 to 536
Ricin725	5' - AGAATAGTTGGGGGAGAC -3'	758 to 775
Ricin937	5' - AATGCTGATGTTGTATG -3'	970 to 987
Ricin1151	5' - CGGGAGTCTATGTGATGA -3'	1184 to 1201
Ricin1399	5' - <u>GCAAATAGTGGACAAGTA</u> -3'	1432 to 1449
Ricin 1627	5' - GGATTGGTGTAGATGTG -3'	1660 to 1677
Ricin1729C	5' - ATAAC TTGCTGTCCTTC -3'	1864 to 1846
Ricin1729C Xba	5' - <u>CGCTCTAGATAACTTGCTGTCCTTC</u>	1864 to 1846

† underlined sequences inserted for subcloning purposes and not included in final preproricin sequences

**Table 2:** Comparative Toxicities to Selected Cell Lines of Ricin and Ricin Provariants

<u>Cell Line</u>	<u>IC50<sub>Ricin</sub></u> <u>(ng/ml)</u>	<u>IC50<sub>pAP214</sub></u> <u>IC50<sub>Ricin</sub></u>	<u>IC50<sub>pAP220</sub></u> <u>IC50<sub>Ricin</sub></u>	<u>IC50<sub>pAP224</sub></u> <u>IC50<sub>Ricin</sub></u>
COS-1	0.1	17	22	150
HT1080	0.5	2.46	2.14	193
9L	10.8	1.3	1.7	32.3
MCF-7 (without estrogen)	0.09	27.8	40	742

**I CLAIM:**

1. A purified and isolated nucleic acid having a nucleotide sequence encoding an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence linking the A and B chains, the heterologous linker sequence containing a cleavage recognition site for a disease-specific protease.
2. The nucleic acid sequence of claim 1 wherein the linker sequence contains a cleavage recognition site recognized by a protease selected from the group consisting of: a cancer associated protease, a viral protease, a fungal protease, and a parasite protease.
3. A nucleic acid sequence of claim 2 wherein the A chain is ricin A chain, abrin toxin A chain, diphtheria toxin A chain, or Domain I of Pseudomonas endotoxin.
4. A nucleic acid sequence of claim 2 wherein the A chain is volkensin toxin A chain, cholera toxin A chain, modeccin toxin A chain or shiga toxin A chain.
5. A nucleic acid sequence of claim 2 wherein the B chain is ricin B chain, abrin toxin A chain, diphtheria toxin B chain, or Domain II of Pseudomonas endotoxin.
- 20 6. A nucleic acid sequence of claim 2 wherein the B chain is volkensin toxin B chain, cholera toxin B chain, modeccin toxin B chain or shiga toxin B chain.
7. A nucleic acid sequence of claim 2 wherein the cleavage recognition site is recognized by a cancer-associated protease which is selected from the group consisting of: cathepsin B, an Epstein-Barr

- 91 -

virus-specific protease, a matrix metalloproteinase, cathepsin L, cathepsin D, urokinase-type plasminogen activator, tissue-type plasminogen activator, human prostate-specific antigen, kallikrein, neutrophil elastase, and calpain.

- 5 8. A nucleic acid sequence of claim 2 wherein the cleavage recognition site is recognized by a parasitic protease which is a *Plasmodium falciparum* protease.
9. A nucleic acid sequence of claim 2 wherein the cleavage recognition site is recognized by viral protease which is selected from  
10 the group consisting of: human cytomegalovirus, human herpes virus, varicella zoster virus, hepatitis A virus, hepatitis C virus, and infectious laryngotracheitis virus.
10. A nucleic acid sequence of claim 2 wherein the cleavage recognition site is recognized by fungal protease which is a *Candida* acid protease.  
15
11. A nucleic acid sequence of claim 2 having the nucleotide sequence according to SEQ ID No. 3; SEQ ID No 5; SEQ ID No 7; SEQ ID No 9; SEQ ID No 11; SEQ ID No 13; SEQ ID No 15; SEQ ID No 17; SEQ ID No 19; SEQ ID No 21; SEQ ID No 23; SEQ ID No 25; SEQ ID No 27;  
20 SEQ ID No 29; SEQ ID No 31; SEQ ID No 33; SEQ ID No 35; SEQ ID No 37; SEQ ID No 39; SEQ ID No 48; SEQ ID No 50; SEQ ID No 52; SEQ ID No 54; SEQ ID No 74; SEQ ID No 77; SEQ ID No 80; SEQ ID No 83; SEQ ID No 86; SEQ ID No 89; SEQ ID No 92; SEQ ID No 95; SEQ ID No 98;  
25 SEQ ID No 101; SEQ ID No 104; SEQ ID No 107; SEQ ID No 110; SEQ ID No 113; SEQ ID No 116; SEQ ID No 119; SEQ ID No 122; or SEQ ID No 125.
12. A plasmid incorporating the nucleic acid of claim 1 to 11.

13. A baculovirus transfer vector incorporating the nucleic acid of claim 1 to 11.
14. A recombinant protein comprising an A chain of a ricin-like toxin, a B chain of a ricin- like toxin and a heterologous linker amino acid sequence, linking the A and B chains, wherein the linker sequence contains a cleavage recognition site for a disease-specific protease.  
5
15. The recombinant protein of claim 14 wherein the linker sequence contains a cleavage recognition site which is recognized by a protease selected from the group consisting of: a cancer, viral, fungal,  
10 and a parasitic protease.
16. A recombinant protein of claim 14 wherein the A chain is ricin A chain, abrin toxin B chain, diphtheria toxin A chain, or Domain I of Pseudomonas endotoxin.
17. A recombinant protein of claim 14 wherein the A chain is volkensin toxin A chain, cholera toxin A chain, modeccin toxin A chain or shiga toxin A chain.  
15
18. A recombinant protein of claim 14 wherein the B chain is ricin B chain, abrin toxin B chain, diphtheria toxin B chain, or Domain II of Pseudomonas endotoxin.
- 20 19. A recombinant protein of claim 14 wherein the B chain is volkensin toxin B chain, cholera toxin B chain, modeccin toxin B chain or shiga toxin B chain.
20. A recombinant protein of claim 14 wherein the cleavage recognition site is recognized by a cancer-associated protease selected

from the group consisting of: cathepsin B, an Epstein-Barr virus-specific protease, a matrix metalloproteinase, cathepsin L, cathepsin D, urokinase-type plasminogen activator, tissue-type plasminogen activator, human prostate-specific antigen, kallikrein, neutrophil 5 elastase, and calpain.

21. A recombinant protein of claim 14 wherein the cleavage recognition site is recognized by a parasitic protease which is a *Plasmodium falciparum* protease.
22. A recombinant protein of claim 14 wherein the cleavage 10 recognition site is recognized by a viral protease which is selected from the group consisting of: human cytomegalovirus, human herpes virus, varicella zoster virus, hepatitis A virus, hepatitis C virus and infectious laryngotracheitis virus.
23. A recombinant protein of claim 14 wherein the cleavage 15 recognition site is recognized by a fungal protease which is a *Candida* acid protease.
24. A recombinant protein of claim 14 having the linker amino acid sequence according to SEQ ID No. 40; SEQ ID No. 41; SEQ ID No. 42; SEQ 20 ID No. 43; SEQ ID No. 44; SEQ ID No. 45; SEQ ID No. 46; SEQ ID No. 55; SEQ ID No. 56; SEQ ID No. 57; SEQ ID No. 58; SEQ ID No. 59; SEQ ID No. 60; SEQ ID No. 61; SEQ ID No. 62; SEQ ID No. 63; SEQ ID No. 64; SEQ ID 25 No. 65; SEQ ID No. 66; SEQ ID No. 67; SEQ ID No. 68; SEQ ID No. 69; SEQ ID No. 70; SEQ ID No. 71; SEQ ID No. 72; SEQ ID No. 75; SEQ ID No. 78; SEQ ID No. 81; SEQ ID No. 84; SEQ ID No. 87; SEQ ID No. 90; SEQ ID No. 93; SEQ ID No. 96; SEQ ID No. 99; SEQ ID No. 102; SEQ ID No. 105; SEQ ID No. 108; SEQ ID No. 111; SEQ ID No. 114; SEQ ID No. 117; SEQ ID 30 No. 120; SEQ ID No. 123; or SEQ ID No. 126.

25. A method of inhibiting or destroying cells affected by a disease, which cells are associated with a protease specific to the disease comprising the steps of:

- (a) preparing a purified and isolated nucleic acid having a nucleotide sequence encoding an A chain of a ricin-like toxin, a B chain of a ricin-like toxin, and a heterologous linker amino acid sequence, linking the A and B chains, wherein the linker sequence contains a cleavage recognition site for the protease;
- 5 (b) introducing the nucleic acid into a host cell and expressing the nucleic acid in the host cell to obtain a recombinant protein comprising an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a linker amino acid sequence;
- 10 (c) suspending the protein in a pharmaceutically acceptable carrier, diluent or excipient, and
- 15 (d) contacting the cells with the recombinant protein.

26. The method of claim 25 where the disease is one of cancer or cells infected with a fungus, virus or parasite.

27. A method of inhibiting or destroying cells affected by a disease, which cells are associated with a protease specific to the disease comprising the step of contacting the cells with a recombinant protein according to any one of claims 14 to 24.

28. A method of treating a disease comprising administering a recombinant protein according to any one of claims 14 to 24 to an animal in need thereof.

25 29. A method of treating a disease comprising administering a nucleic acid molecule according to any one of claims 2 to 11 to an animal in need thereof.

30. A method of treating a mammal with cancer or infected with a fungus, virus or parasite, comprising the steps of preparing a recombinant protein of claim 14 wherein the linker sequence contains a cleavage recognition site for a cancer, fungal, viral or parasitic protease  
5 and administering the protein to the mammal.

31. A process for preparing a pharmaceutical for treating a mammal with cancer, fungal infection, viral infection or parasitic infection, comprising the steps of :

(a) preparing a purified and isolated nucleic acid having a  
10 nucleotide sequence encoding an A chain of a ricin-like toxin, a B chain of a ricin-like toxin, and a heterologous linker amino acid sequence, linking the A and B chains, wherein the linker sequence contains a cleavage recognition site for a cancer, viral or parasitic protease;

(b) introducing the nucleic acid into a host cell and expressing  
15 the nucleic acid in the host cell to obtain a recombinant protein comprising an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a linker amino acid sequence;

(c) suspending the protein in a pharmaceutically acceptable carrier, diluent or excipient.

20 32. A use of a recombinant protein according to any one of claims 14 to 24 to treat a disease.

33. A use of a nucleic acid molecule according to any one of claims 1 to 11 to treat a disease.

34. A pharmaceutical composition for treating cancer or a fungal, or  
25 viral, or parasitic infection in an animal comprising the recombinant protein of claim 14 and a pharmaceutically acceptable carrier, diluent or excipient.

- 96 -

35. A pharmaceutical composition for treating cancer or a fungal, or viral, or parasitic infection in an animal comprising the nucleic acid molecule of claim 2 and a pharmaceutically acceptable carrier, diluent or excipient.

1/254

FIGURE 1Complete Sequence of Baculovirus  
Transfer Vector, pVL1393

ID PVL1393 preliminary; circular DNA; SYN;  
 9632 BP.  
 XX  
 AC IG1137;  
 XX  
 DT 01-FEB-1993 (Rel. 7, Created)  
 DT 01-JUL-1995 (Rel. 12, Last updated, Version  
 1)  
 XX  
 DE E. coli plasmid vector pVL1393 - complete.  
 XX  
 KW cloning vector.  
 XX  
 OS Cloning vector  
 OC Artificial sequences; Cloning vehicles.  
 XX  
 RN [1]  
 RC p2Bac from baculovirus  
 RC p2Blue from p2Bac  
 RC pBlueBac from AcNPV  
 RC pBlueBac2 from AcNPV  
 RC pBlueBacIII from AcNPV  
 RC pBlueBacHisA from AcNPV  
 RC pBlueBacHisB from AcNPV  
 RC pBlueBacHisC from AcNPV  
 RC pVL1392, pVL1393 from pAc360  
 RA ;  
 RT ;  
 RL The Digest 5:2-2(1992).  
 XX  
 CC NM (pVL1393)  
 CC CM (yes)  
 CC NA (ds-DNA)  
 CC TP (circular)  
 CC ST ()  
 CC TY (plasmid)  
 CC SP (British  
 Biotechnology) (Invitrogen)  
 CC HO (E.coli NM522) (E.coli  
 INValphaF') (insect)  
 CC CP ()  
 CC FN (expression) (transfer)  
 CC SE ()  
 CC PA (pAC360)  
 CC BR (pVL1392)  
 CC OF ()  
 CC OR ()  
 XX  
 FH Key Location/Qualifiers  
 FH

2/254

FIGURE 1 (Cont'd)

```

FT misc_feature 0..0
FT /note="1. pAc360, ori/amp/AcMNPV
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FT -> pVL1393 9632bp"
FT transposon 0..0
FT /note="TRN AcMNPV"
FT misc_binding 868..868
FT /note="SIT SacII"
FT misc_binding 1395..1395
FT /note="SIT ApaI"
FT misc_binding 1901..1901
FT /note="SIT XbaI"
FT promoter 0..0
FT /note="PRO AcMNPV polyhedrin gene"
FT misc_binding 0..0
FT /note="MCS
FT BamHI-SmaI-XbaI-EcoRI-NotI-XmaIII-PstI-
BgIII."
FT rep_origin 0..0
FT /note="ORI E. coli pMB1 (ColE1 and
pBR322)*
FT CDS complement(0..0)
FT /note="ANT E. coli beta-lactamase gene
(bla)
FT ampicillin resistance gene (apr/amp)"

XX

SQ Sequence 9632 BP; 2602 A; 2122 C; 2176 G; 2732 T; 0
other;
aagctttact cgtaaaagcga gttgaaggat catatttagt tgcgtttatg
agataagatt gaaagcacgt gtaaaatgtt tcccgccgt tgccacaact
atttacaatg cggccaagtt ataaaaagatt ctaatctgat atgttttaaa
acaccttgc ggcccggagtt gtttgcgtac gtgactagcg aagaagatgt
gtggaccgca gaacagatag taaaacaaaa ccctagtatt ggagcaataa
tcgatTTAAC caacacgtct aaatattatg atggtgtgca tttttgcgg
gcgggcctgt tataaaaaaa aattcaagta cctggccaga ctggccgccc
tgaaagcata gttcaagaat ttattgacac ggtaaaaagaa ttacagaaaa
agtgtcccggtt catgttgggtt ggcgtgcact gcacacacgg tattaatcgc
accggttaca tgggtgtgcag atattaatg cacaccctgg gtattgcgcc
gcaggaagcc atagatagat tcgaaaaaagc cagaggtcac aaaattgaaa
gacaaaatta cgttcaagat ttattaattt aattaatatt atttgcattc
tttaacaaat actttatcct atttcaaatt ttttgcgtt cttccagcga
acccaaacta tgcttcgctt gctccgttta gcttgttagcc gatcagtggc
gttggccaa tcgacggtag gattaggccg gatattctcc accacaatgt
tggcaacgtt gatgttacgt ttatgtttt ggtttccac gtacgtctt
tggccggtaa tagccgtaaa cgttagtgcgg tcgcgtca cgcacaaacac
cgatgtttt cgttgcgttccg cgggttattt aaccgcgcga tccgacaaat
ccaccacttt ggcaactaaa tcgggtgaccc ggcgtcttt tttctgcatt
atttgcgttt tctttgcatt ggtttccgg aagccgggtt acatgcgggtt
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tgtccttgcatt ggcaacgtatg cgttcaataaa actcttgcattt tttaacaagtt
tcctcggttt tttgcgtccac caccgcttgc agcgcgtttt tttgcgtctcc tcctccgtt
aatgtcgca atcagcttag tcaccaactg tttgcgttcc tcctccgtt
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tcttctaaaa gccattcttgc taattctatg tcgtaaaggca atttggactt

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3/254

**FIGURE 1 (Cont'd)**

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**FIGURE 1 (Cont'd)**

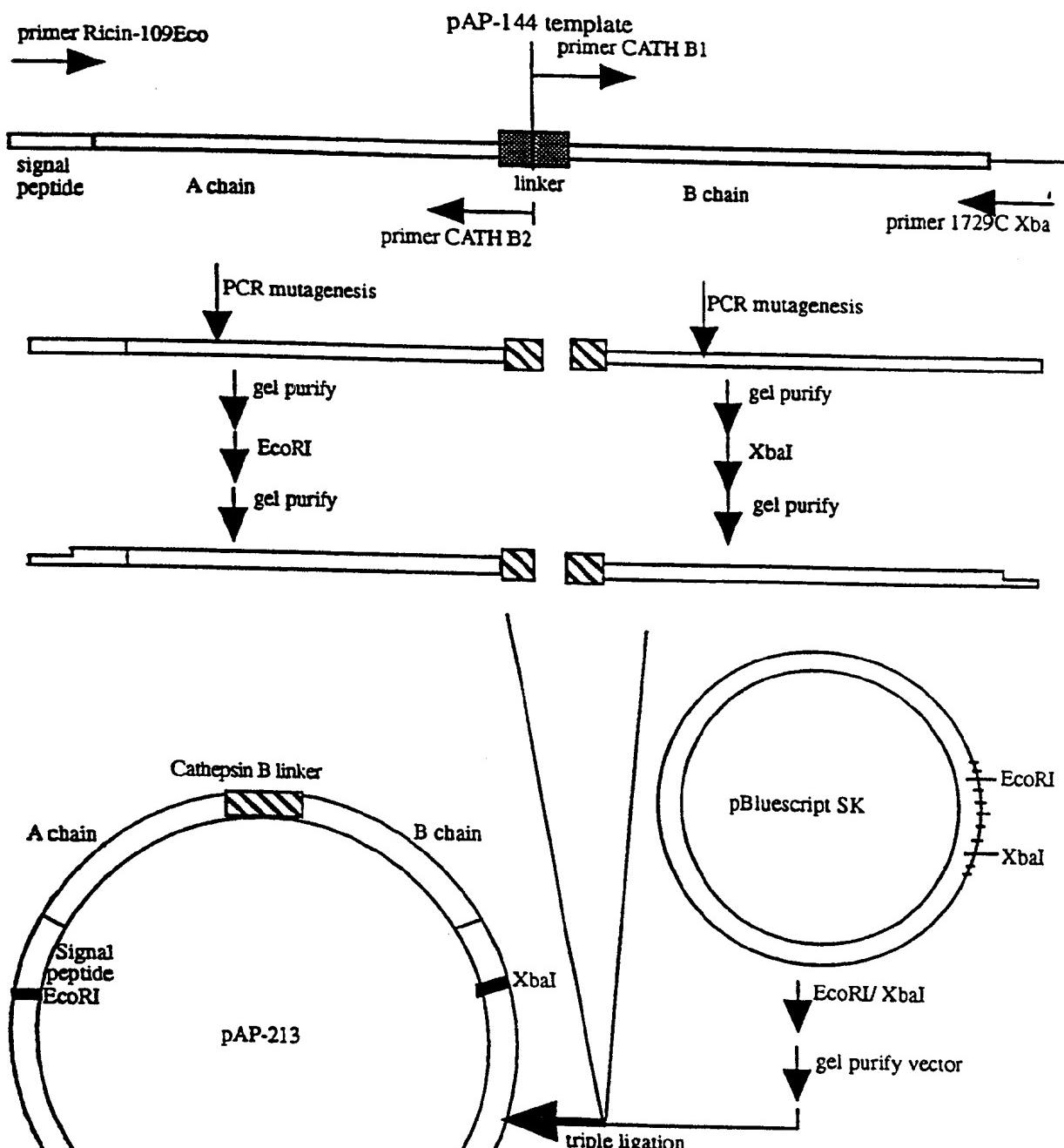
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 gatacatatt tgaatgtatt tagaaaaata aacaaatagg ggttccgcgc  
 acatttcccc gaaaagtggc acctgacgac taagaaacca ttattatcat  
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**FIGURE 1 (Cont'd)**

## FIGURE 1 (Cont'd)

6/254

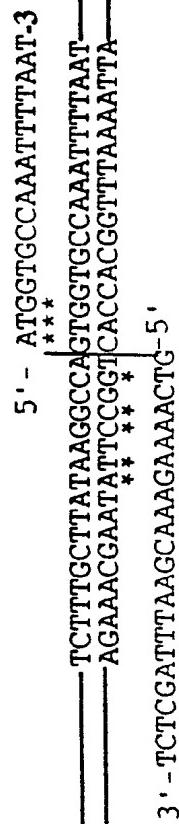
7/254

**FIGURE 2A**

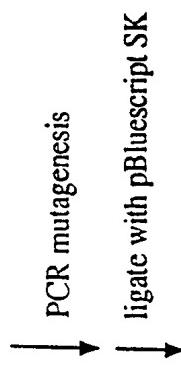
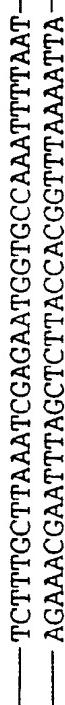
8/254

**FIGURE 2B****WT preprorocin linker**

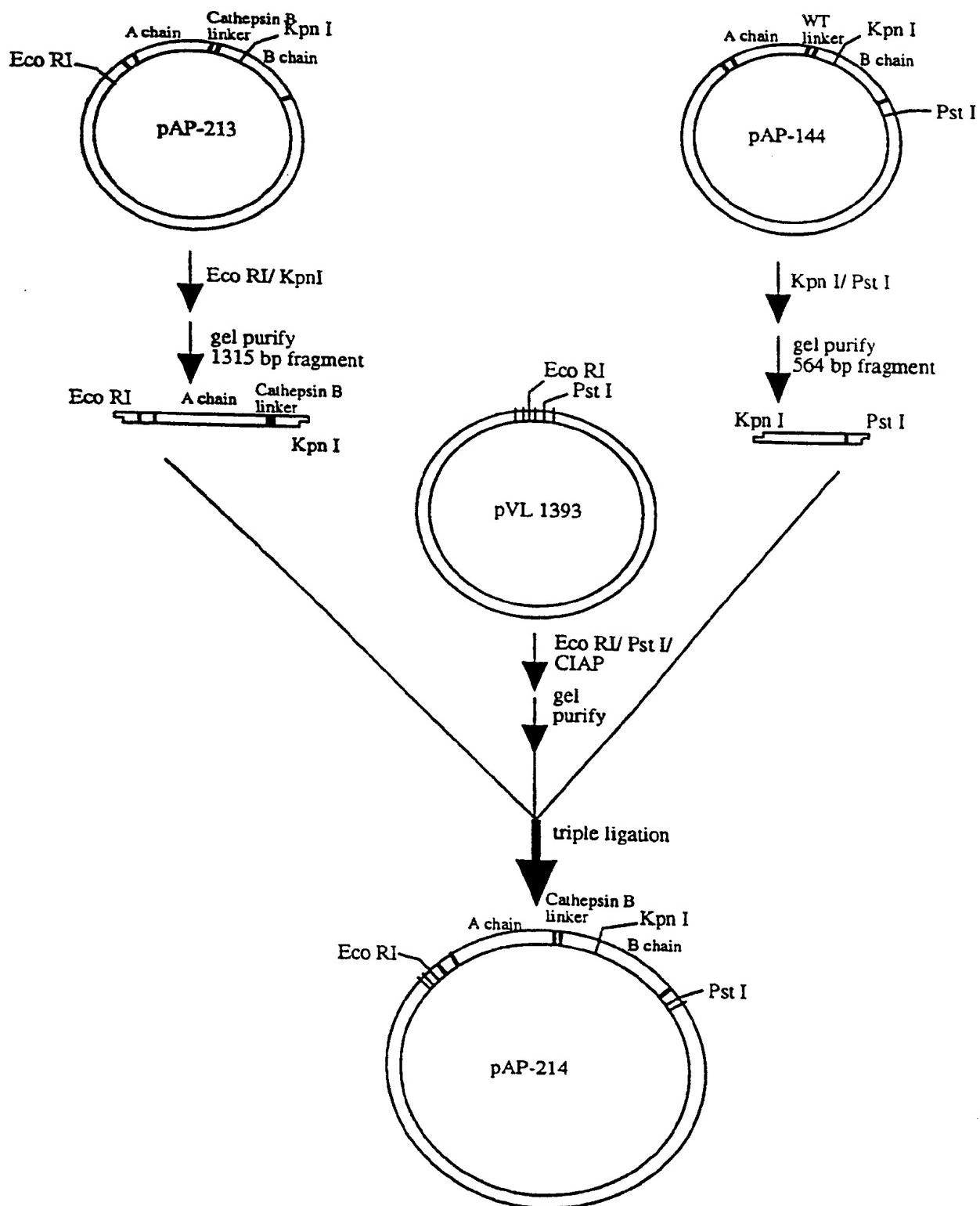
primer CATH-B1



primer CATH-B2

**pAP 213 linker  
(Cathepsin-B variant)**

9/254

**FIGURE 2C**

10/254

**FIGURE 2D**

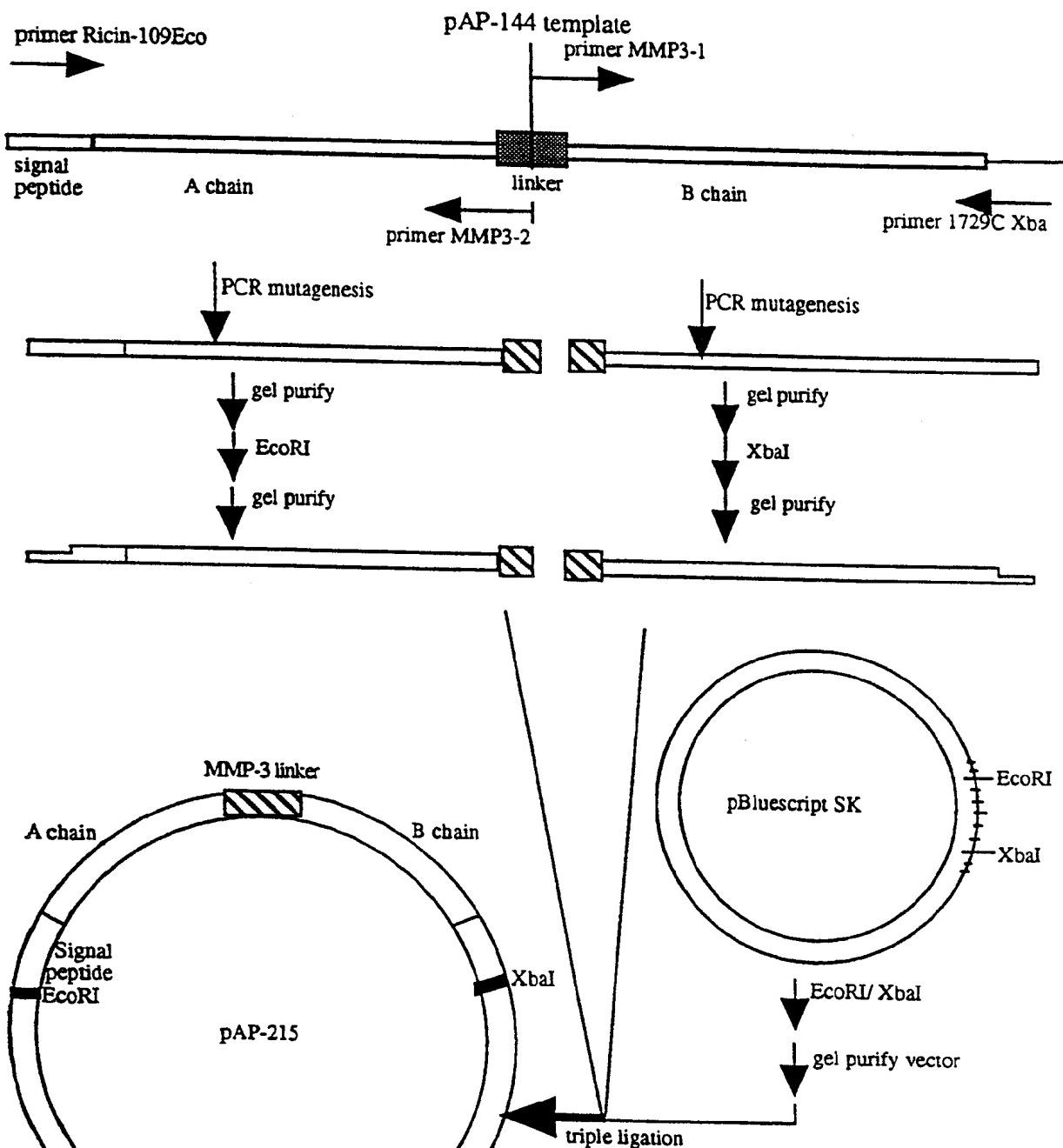
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51 GGCAACATGGCTTGTGTTGGATCCACCTCAGGGTGGCTTCACATTAG				
CCGTTGTACCGAAAACCTAGGTGGAGTCCCACCAGAAAAGTGTAAATC				
101 AGGATAACAACATATTCCCCAACAAACACCAATTATAAACTTTACACAA				
TCCTATTGTTGTATAAGGGTTGTTATGGGTTAATATTGAAATGGTGT				
151 GCGGGTGCCACTGTGCAAAGCTACACAAACCTTATCAGAGCTGTTGCCG				
CGCCCACGGTGACACGTTGATGTGTTGAATAGTCTCGACAAGCGCC				
201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCAAGTGTGCCAA				
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT				
251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACCTCTCA				
TGTCTCAACCAAACGGATATTGGTTGCCAAATAAAATCAACTTGAGAGT				
301 AATCATGCAGAGCTTCTGTACATTAGCGCTGGATGTACCAATGCATA				
TTAGTACGTCTCGAAAGACAATGTAATCGCACCTACAGTGGTTACGTAT				
351 TGTGGTCGGTACCGTGTGGAAATAGCGCATATTTCTTCATCCTGACA				
ACACCAGCCGATGGCACGACCTTATCGCGTATAAAGAAAGTAGGACTGT				
401 ATCAGGAAGATGCAGAACATCACTCATCTTTCACTGATGTTCAAAAT				
TAGTCCTTCTACGTCTCGTTAGTAGAAAGTGAACACTACAAGTTTA				
451 CGATATACATTGCCCTTGGTGGTAATTATGATAGACTTGAACAACTTGC				
GCTATATGTAAGCGAAACCACCATTAATACTATCTGAACTTGTTGAACG				
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG				
ACCATTAGACTCTCTTTATAGCTCAACCCTTACCGAGGTGATCTCCCTCC				
551 CTATCTCAGCGCTTTATTACAGTACTGGTGGCACTCAGCTTCAACT				
GATAGAGTCGCGAAATAATGTCATGACCACCGTGAGTCGAAGGTTGA				
601 CTGGCTCGTCCCTTATAATTGCACTCCAAATGATTTAGAAGCAGCAAG				
GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTGTTC				
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA				
TAAGGTTATATAACTCCCTTTACCGTGCTTAATCCATGTTGGCCT				
701 GATCTGCACCAAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA				
CTAGACGTGGCTAGGATCGCATTATGTGAACCTTATCAACCCCTCT				
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGGAGCCTTGCTAGTCCAAT				
GAAAGGTGACGTTAAGTCTCAGATTGGTCTCGGAAACGATCAGGTTA				
801 TCAACTGCAAAGACGTAATGGTCCAATTCACTGAGTGTACGATGTGAGTA				
AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT				
851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA				
ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACCGTGAGGTTG				
901 TCGTCACAGTTCTTGCTTAAATCGAGAATGGTGCCTAAATTAAATGC				
AGCAGTGTCAAAAGAACGAAATTAGCTCTTACCAACGGTTAAATTACG				

11/254

**FIGURE 2D (CONT'D)**

951 TGATGTTGTATGGATCCTGAGCCCATACTGCCTATCGTAGGTCGAAATG  
     ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
     CAGATACACAACATACTACATCCCTACCTCTAAGGTGTCCTTGCGTTAT  
 1051 CAGTTGTGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
     GTCAACACCGGTACGTTAGATTATGTCTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAGTGTAACTACTTACG  
     CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCGGGAGTCTATGTGATGATCTATGATTGCAAACTGCTGCA  
     CCATGTCAGGCCCTCAGATACTACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAATCC  
     TGACTACGGTGGCGACCGTTATACCTTACCTTGTTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTTACAGCGACATCAGGGAACAGTGGTACCAACAC  
     GTCTAGATCAGATAACGTCGCTGTAGTCCTGTCACCAGGGTGTG  
 1301 TTACAGTGAAACCAACATTATGCCGTTAGTCAGGTTGGCTTCTACT  
     AATGTCACGTTGGTTGTAATACGGCAATCAGTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGCTGTG  
     TTATTATGTGTTGGAAAACAATGTTGTAACAACCCGATATACCAACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
     GAACGTTCGTTATCACCTGTCATACTATCCTGACATCGTCACTTT  
 1451 AGGCTGAACAAACAGTGGCTTTATGCAGATGGTCAATACGTCCTCAG  
     TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTACAAGTATTCTAATATAACGGAAACAGT  
     GTTTGGCTCTATTAACGGAATGTTACTAAGATTATATGCCCTTGTCA  
 1551 TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
     ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA  
 1601 TCAAGAATGATGGAACCATTAAATTGTTAGTGTAGTGGATGGTGTAGAT  
     AGTTCTTACTACCTGGTAAATTAACATATCACCTAACCAACAACTCA  
 1651 GTGAGGCAGTCGGATCCGAGCCTAAACAAATCATTCTTACCCCTCTCCA  
     CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
 1701 TGGTGACCCAAACCAAATATGGTACCAATTGTTAGTGTAGACAGATTACT  
     ACCACGGGTTGGTTATACCAATGGAATAAAACTATCTGTCTAATGA  
 1751 CTCTTGCAAGTGTGTCCTGCCATGAAAATAGATGGCTAAATAAAAAA  
     GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATTTT  
 1801 GGACATTGTAATTTGTAACGTAAAGGACAGCAAGTTATATCGAATTCC  
     CCTGTAACATTAAAACATTGACTTCCCTGTCGTTCAATATAGCTTAAGG  
 1851 TGCAG  
     ACGTC

12/254

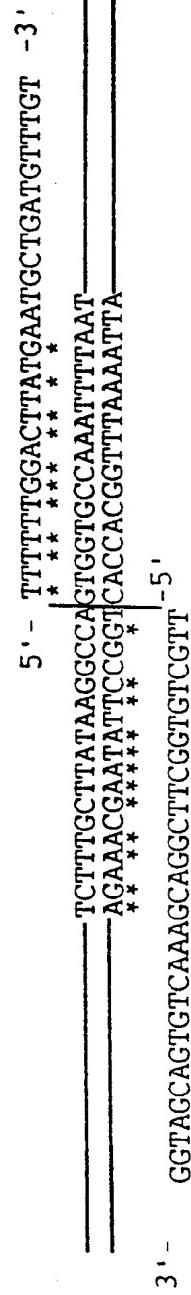
**FIGURE 3A**

13/254

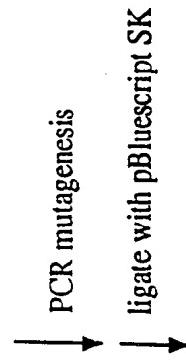
**FIGURE 3B**

WT preprotein linker

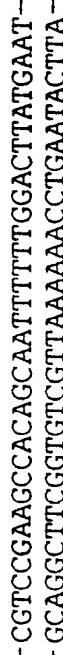
primer MMP3-1



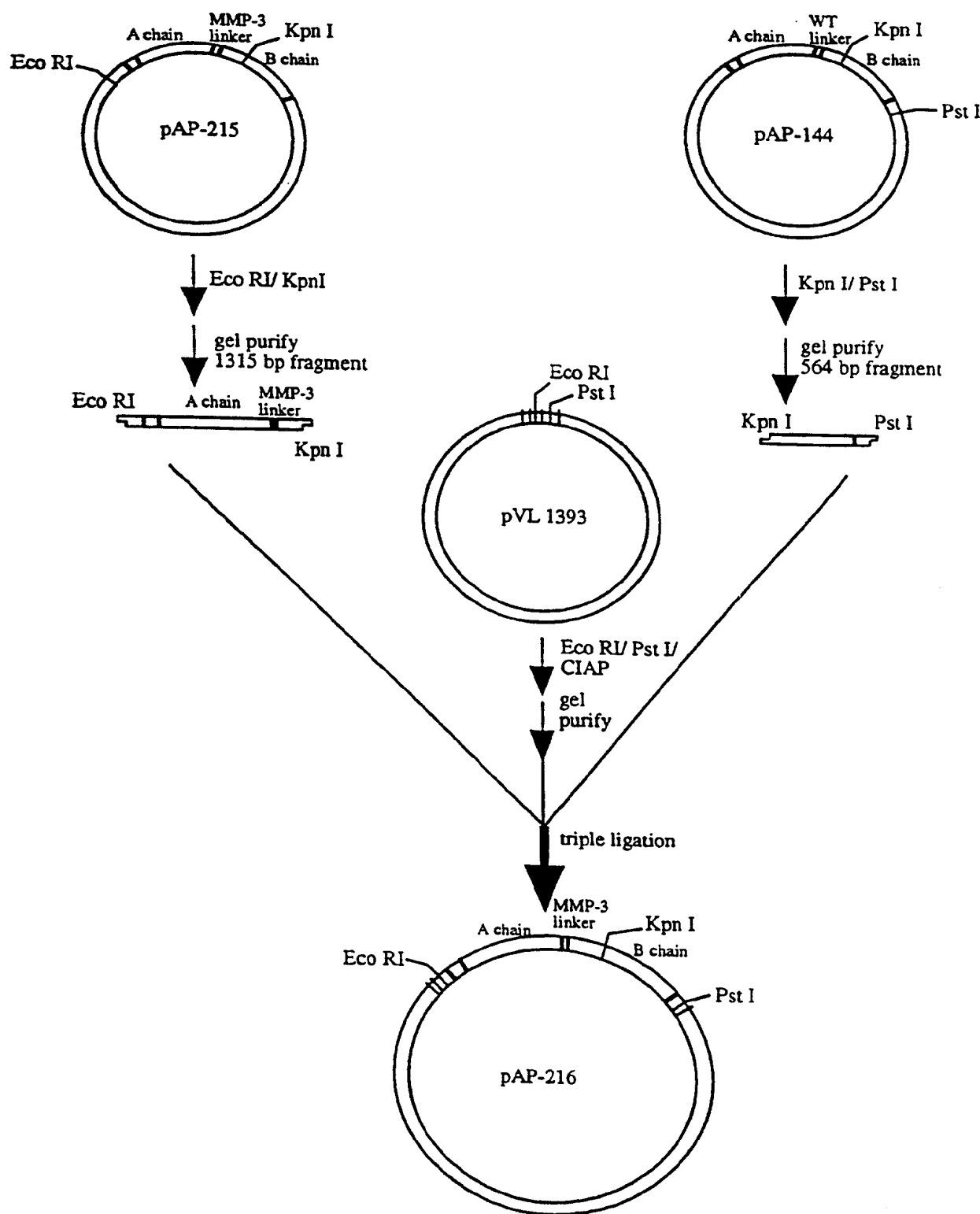
primer MMP3-2



pAP 215 linker  
 (MMP-3 variant)



14/254

**FIGURE 3C**

15/254

FIGURE 3D

10                  20                  30                  40                  50

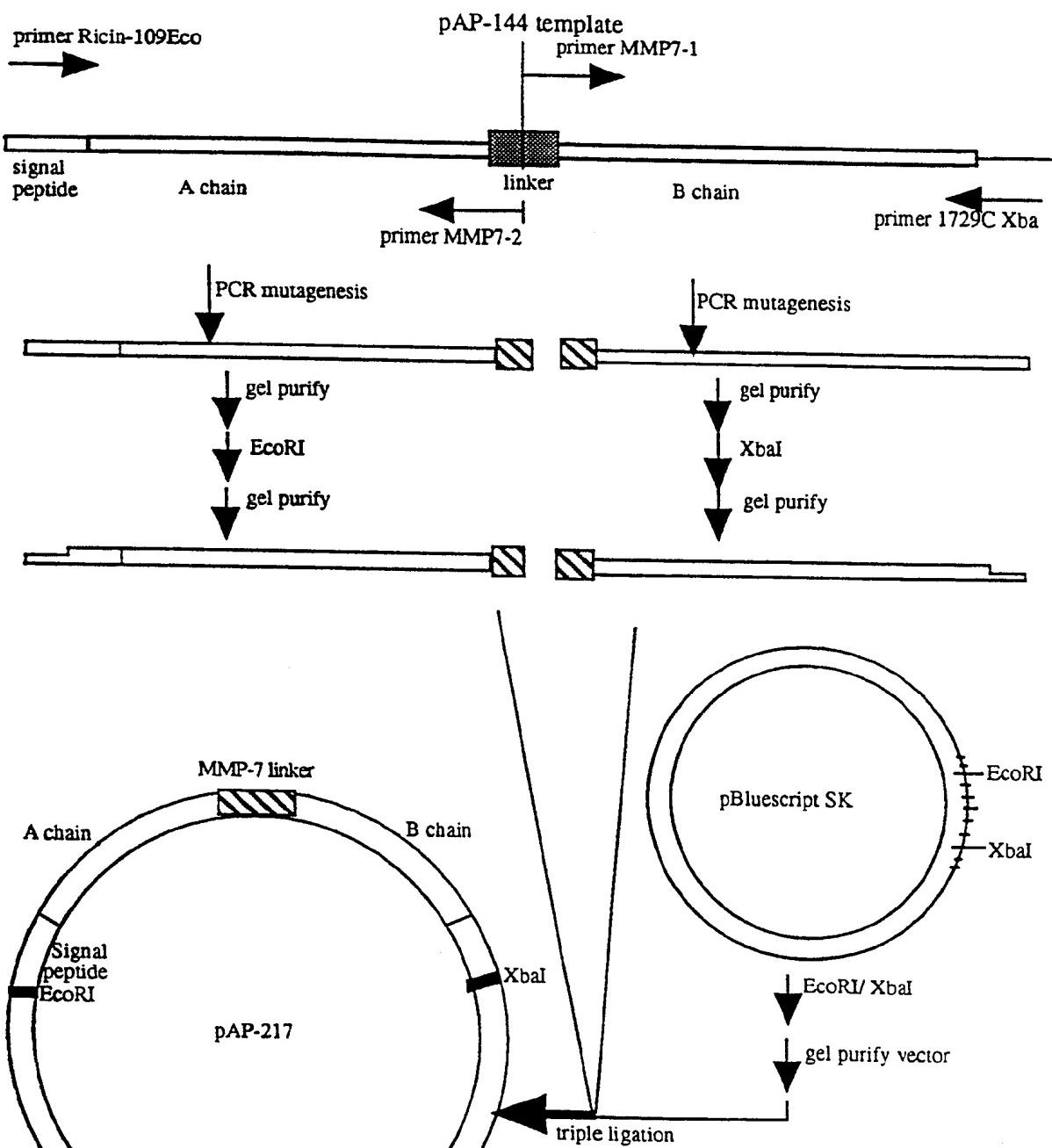
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 51 GGCAACATGGCTTGGATCCACCTCAGGGTGGCTTCACATTAG  
 CGTTGTACCGAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC  
 101 AGGATAACAACATATTCCCCAACAAACCAATTATAAACTTTACCA  
 TCCTATTGTTGATAAGGGTTGTTATGGTTAATATTGAAATGGTGT  
 151 GCGGGTGCCACTGTGCAAAGCTACACAAACCTTATCAGAGCTGTTCGCGG  
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 201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATAACCAAGTGTGCCAA  
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 401 ATCAGGAAGATGCAGAACAACTCATCTTCACTGATGTTAAAAT  
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 GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA  
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 TAAGGTTATATAACTCCCTTTACCGGTGCTCTTAATCCATGTTGGCCT  
 701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA  
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 851 TATTAATCCCTATCATAGCTCTCATGGTGATAGATGCGCACCTCCACCA  
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 901 TCGTCACAGTTCTCGTCCGAAGGCCACAGCAATTGGACTTATGAATGC  
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16/254

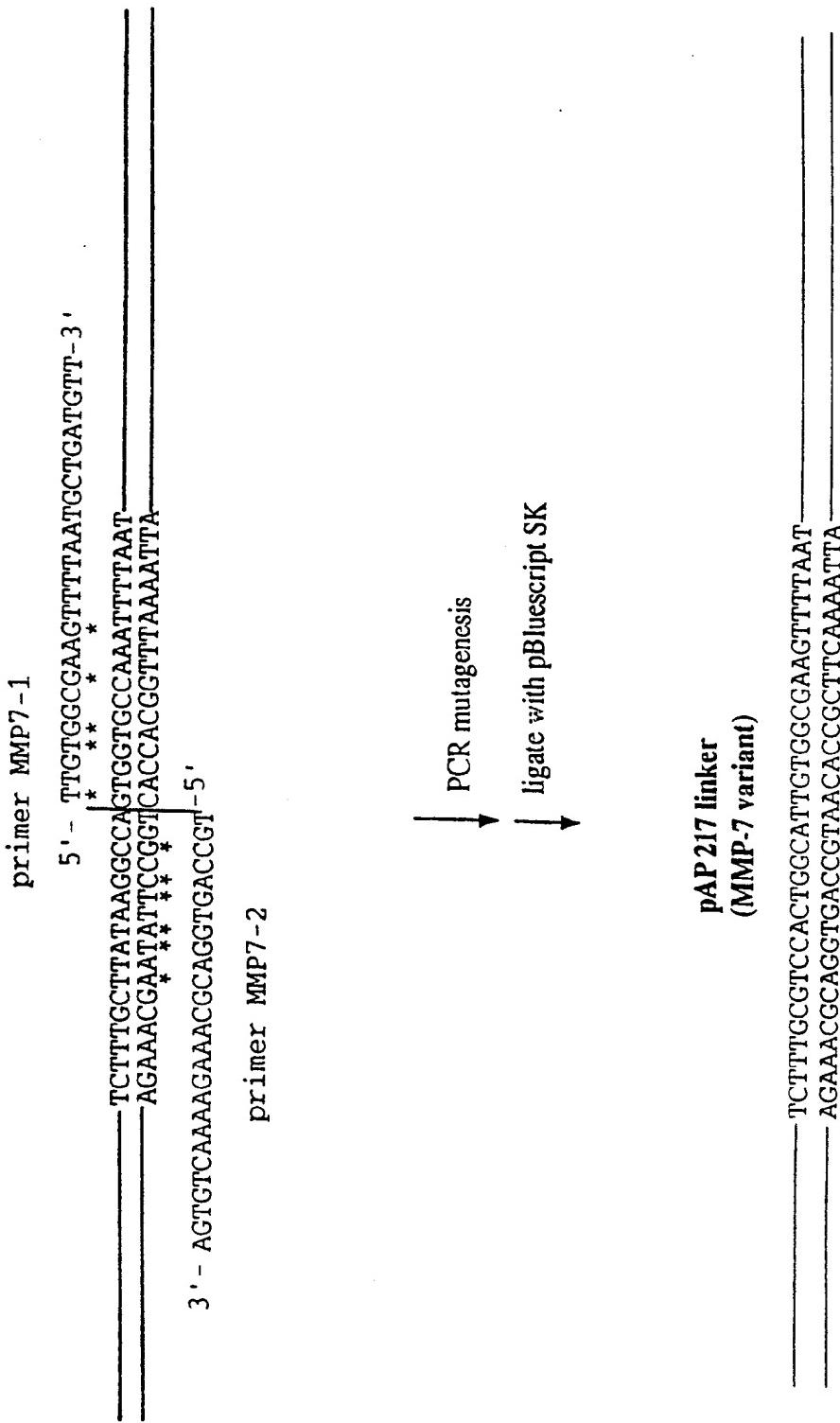
FIGURE 3D (CONT'D)

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 1051 CAGTTGTGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
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 1151 GGTACAGTCGGGAGTCTATGTGATGATCTATGATTGCAAACTGCTGCA  
     CCATGTCAGGCCCTCAGATACACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCACATCATAATCC  
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 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
     GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTCACCATGGTGTG  
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 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGCTGTG  
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     GAACGTTCGTTATCACCTGTTACCTATCTCCTGACATCGTCACTT  
 1451 AGGCTGAACAAACAGTGGCCTTTATGCAGATGGTCAATACGTCTCAG  
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 1501 CAAAACCGAGATAATTGCCCTACAAGTGATTCTAATATAACGGAAACAGT  
     GTTTGGCTCTATTAACGGAAATGTTCACTAAAGATTATATGCCCTTGTCA  
 1551 TGTAAAGATCCTCTCTGTTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
     ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTGCTACCTACA  
 1601 TCAAGAATGATGGAACCATTAAATTGTTAGTGTAGTGGATGGTAGAT  
     AGTTCTTACTACCTGGTAAATTAACATATCACCTAACCAACAACTCA  
 1651 GTGAGGCAGTCGGATCCGAGCCTAAACAAATCATTCTTACCCCTCTCCA  
     CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
 1701 TGGTGAACCAAACAAATATGGTACCATATTGGATAGACAGACATTACT  
     ACCACTGGGTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA  
 1751 CTCTTGCACTGTGTTGCTCTGCCATGAAAATAGATGGCTAAATAAAAAA  
     GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATTTT  
 1801 GGACATTGTAATTGGTAACGTAAAGGACAGCAAGTTATATCGAATTCC  
     CCTGTAACATTAAAACATTGACTTCCTGTCGTTCAATATAGCTTAAGG  
 1851 TGCAG  
     ACGTC

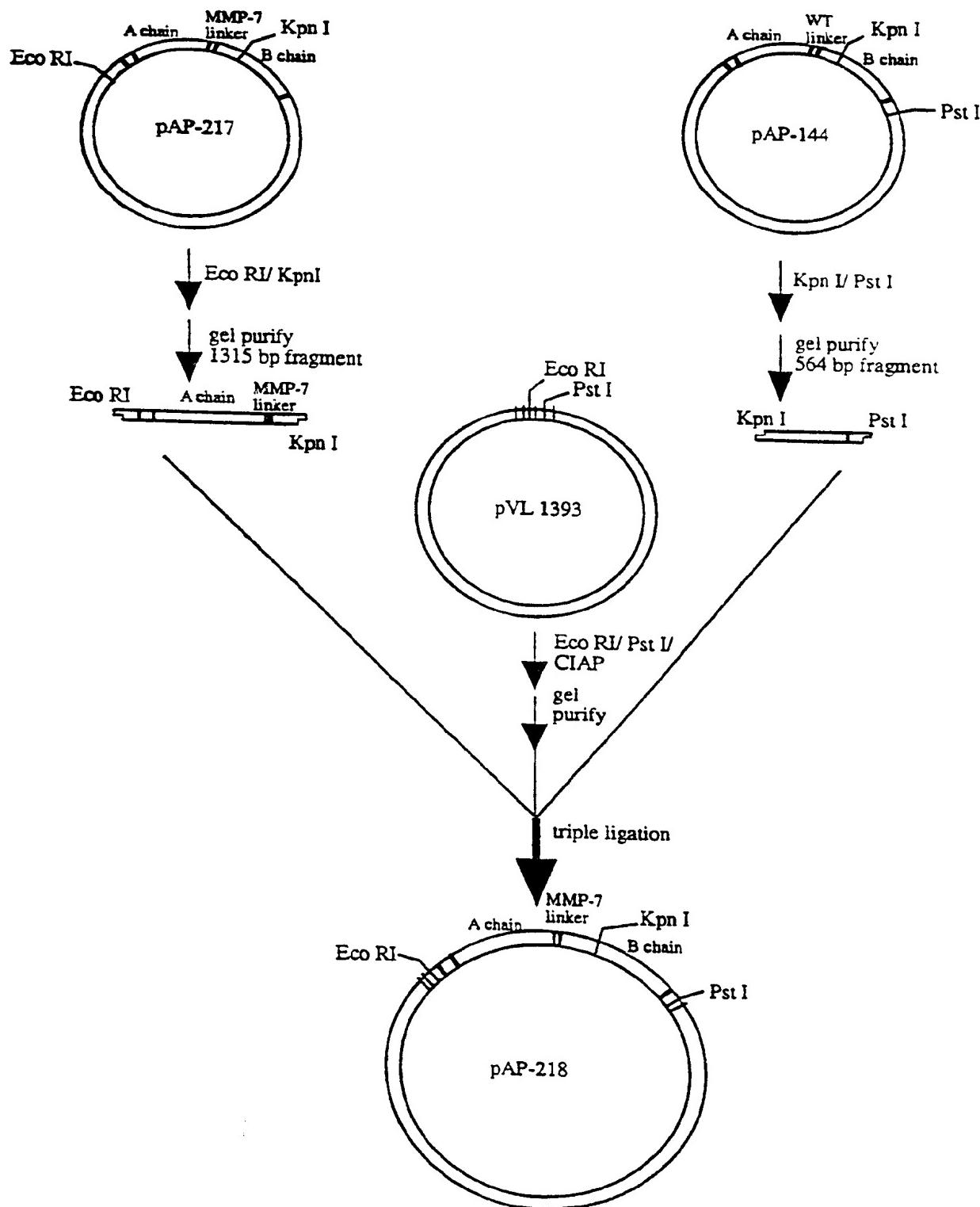
17/254

**FIGURE 4A**

18/254

**FIGURE 4B****WT preprorocin linker**

19/254

FIGURE 4C

20/254

FIGURE 4D

10            20            30            40            50

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1  GAATTCATGAAACCAGGGAGGAATACTATTGTAATATGGATGTATGCAGT
   CTTAAGTACTTGGCCCTCCTTATGATAACATTATACCTACATACGTCA

51  GGCAACATGGCTTGTGATCCACCTCAGGGTGGCTTCACATTAG
   CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC

101 AGGATAACAACATATTCCCCAACAAATACCCATTATAAACTTACCA
   TCCTATTGTTGATAAGGGTTGTTATGGTTAATATTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAAACCTTATCAGAGCTGTTCGCGG
   CGCCCACGGTGACACGTTGATGTGTTGAAATAGTCTCGACAAGCGCC

201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATAACCAAGTGTGCCAA
   AGCAAATTGTTGACCTCGACTACACTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACCTCTCA
   TGTCTCAACCAAACGGATATTGGTGCCTAAATAACTCAACTGAGAGT

301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
   TTAGTACGTCTCGAAAGACAATGTAATCGCACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTCTTCATCCTGACA
   ACACCAAGCCGATGGCACGACCTTATCGGTATAAAAGAAAGTAGGACTGTT

401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTCACTGATGTTAAAAT
   TAGTCCTCTACGTCTTGTAGTAGAAAAGTGAACACAAGTTTA

451 CGATATACACATTGCCCTTGGTGGTAATTATGATAGACTTGAACAACCTGC
   GCTATATGTAAGCGAAACCACCATTAATACTATCTGAACCTGGTTGAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCACTAGAGGGAGG
   ACCATTAGACTCTTTATAGCTCAACCCTTACCAAGGTGATCTCCTCC

551 CTATCTCAGCGCTTATTACAGTACTGGTGGCACTCAGCTTCAACT
   GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGGTTGA

601 CTGGCTCGTCTTATAATTGATCCAAATGATTCAGAAGCAGCAAG
   GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTGTTC

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
   TAAGGTTATATAACTCCCTTTACCGTGCTTAAATCCATGTTGGCCT

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA
   CTAGACGTGGCTAGGATCGCATTAAATGTAACCTTATCAACCCCTCT

751 CTTTCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCAAAT
   GAAAGGTGACGTTAACGTTAGTCTCAGATTGGTCTCGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTCCAAATTCAAGGTGAGTGTACGATGTGAGTA
   AGTTGACGTTCTGCATTACCAAGGTTAACGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
   ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACCGGTGGAGGTGGT

901 TCGTCACAGTTCTTGCCTCCACTGGCATTGTGGCGAAGTTTAATGC
   AGCAGTGTCAAAAGAAACGCAGGTGACCGTAACACCGCTCAAAATTACG

951 TGATGTTGTATGGATCCTGAGCCCAGTGCGTATCGTAGGTCGAAATG

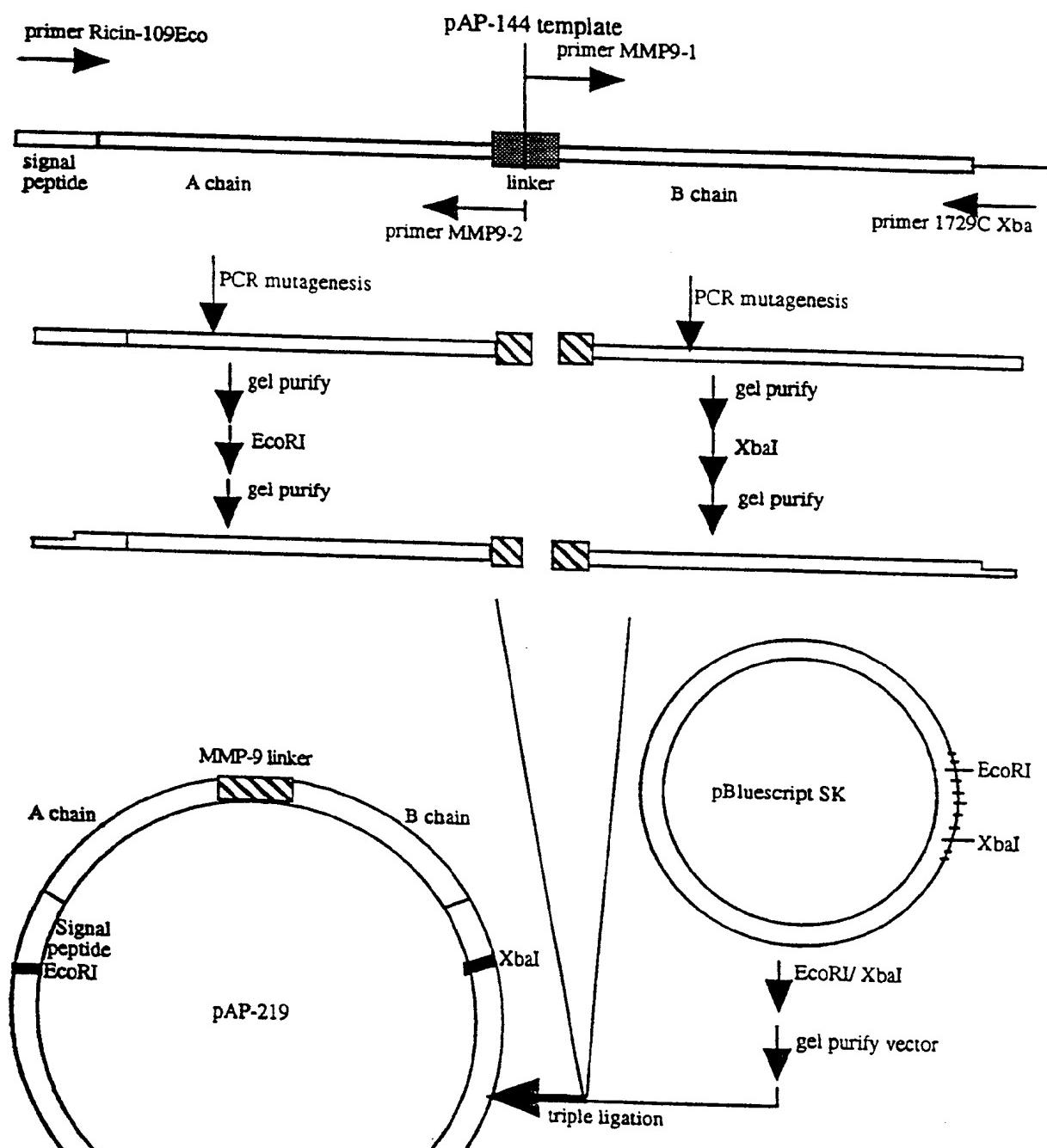
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21/254

**FIGURE 4D (CONT'D)**

ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
 CAGATACACAACATAACATCCCTACCTTCTAAGGTGTTGCCCTTGCGTTAT  
 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTACGATTATGTCTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGAAAGTGTAACTACTAACG  
 CTTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAAATACTGCTGCA  
 CCATGTCAGGCCCTCAGATAACACTAGATAACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCACATCAAATCC  
 TGACTACGGTGGCGACCCTTATACCCATTACCTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTTACAGCGACATCAGGGAACAGTGGTACCAACAC  
 GTCTAGATCAGATCAAATCGTCGCTGAGTCCCTGTCACCATGGTGTG  
 1301 TTACAGTGCAAACCAACATTATGCCGTTAGTCAGGTTGGCTTCTACT  
 AATGTCACGTTGGTTGTAACAGGCAATCAGTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
 TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATAACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
 GAACGTTCGTTATCACCTGTTACACCTATCTCCTGACATCGTCACCTT  
 1451 AGGCTGAACAAACAGTGGCTTTATGCAGATGGTCAATACGTCCAG  
 TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGATTCTAATATAACGGAAACAGT  
 GTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTGTCA  
 1551 TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
 ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTGCTACCTACA  
 1601 TCAAGAATGATGGAACCAATTAAATTGTATAGTGGATTGGTGTAGAT  
 AGTTCTTACTACCTGGTAAATTAAACATATCACCTAACCAACAACTCA  
 1651 GTGAGGCAGATCGGATCCGAGCCTTAAACAAATCATTCTTACCCCTCCA  
 CACTCCGCTAGGCTCGGAATTGTTAGTAAGAAATGGAGAGGT  
 1701 TGGTGACCCAAACCAATATGGTACCAATTATTTGATAGACAGATTA  
 ACCACTGGGTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA  
 1751 CTCTTGCAGTGTGTGTCCTGCCATGAAAATAGATGGCTAAATAAAAAA  
 GAGAACGTACACACACAGGACGGTACTTTATCTACCGAATTATTTT  
 1801 GGACATTGTAATTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC  
 CCTGTAACATTAAAACATTGACTTCCTGTCGTTCAATATAGCTTAAGG  
 1851 TGCAG  
 ACGTC

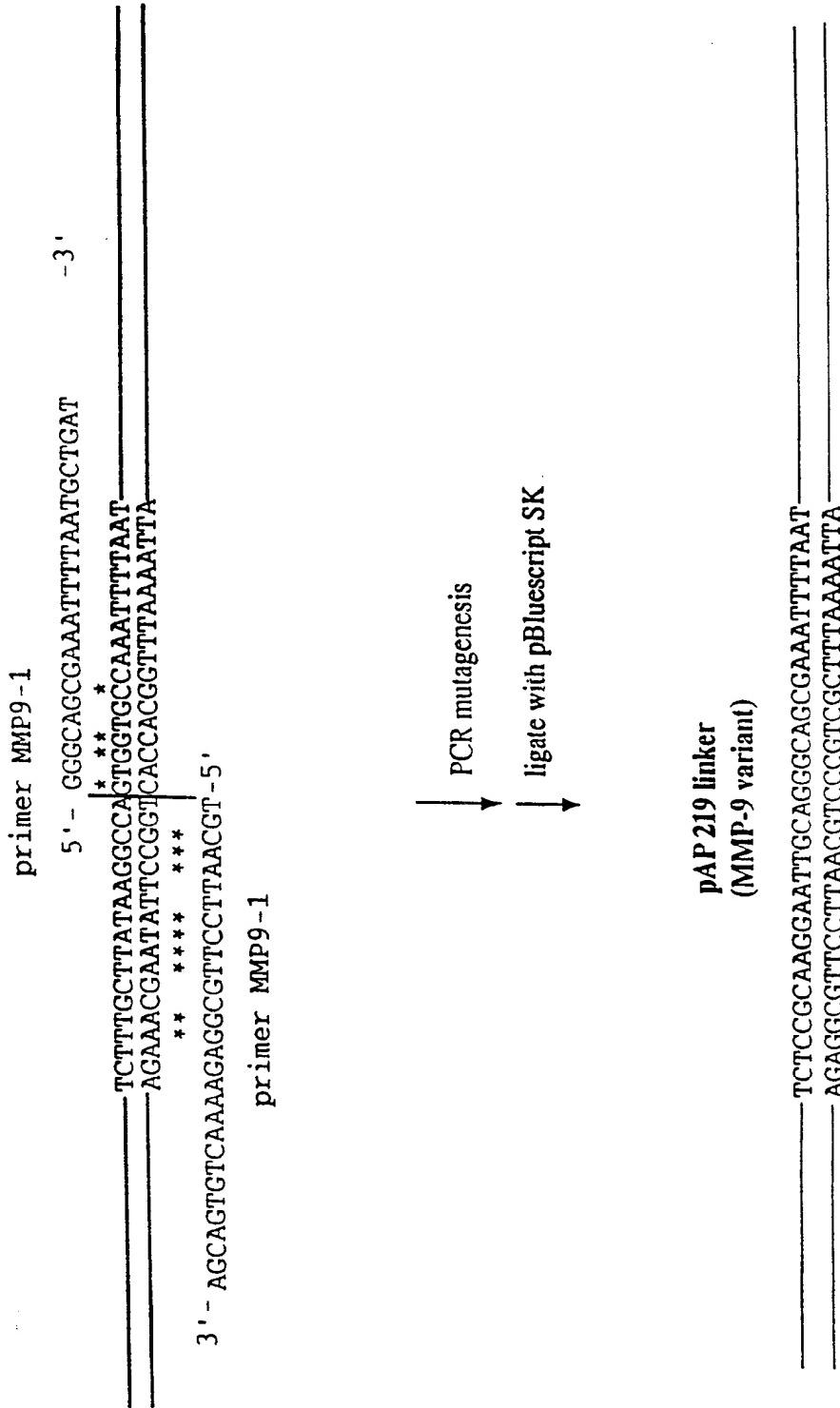
22/254

FIGURE 5A

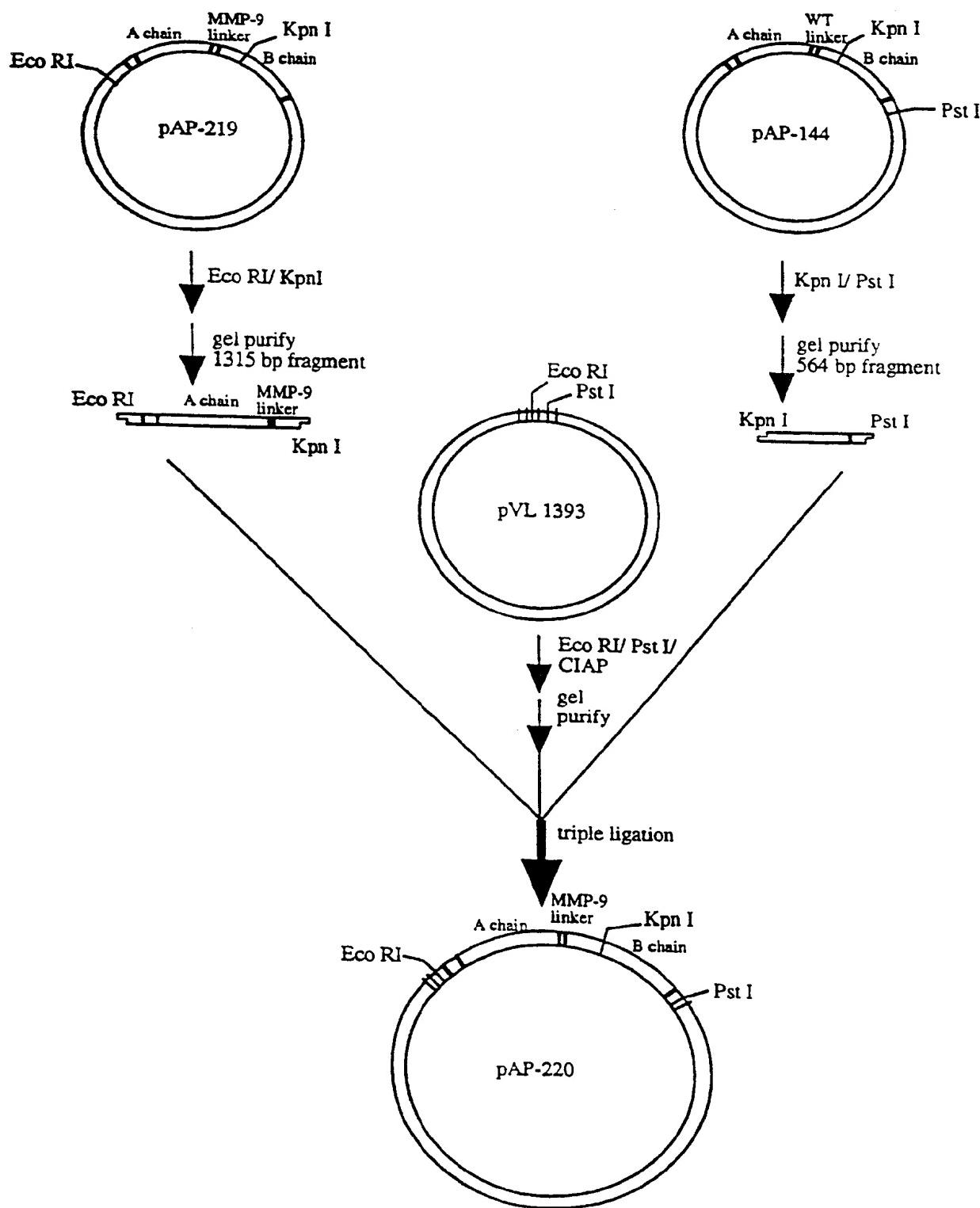
23 / 254

## FIGURE 5B

WT prorocin linker



24/254

FIGURE 5C

25/254

FIGURE 5D

10            20            30            40            50

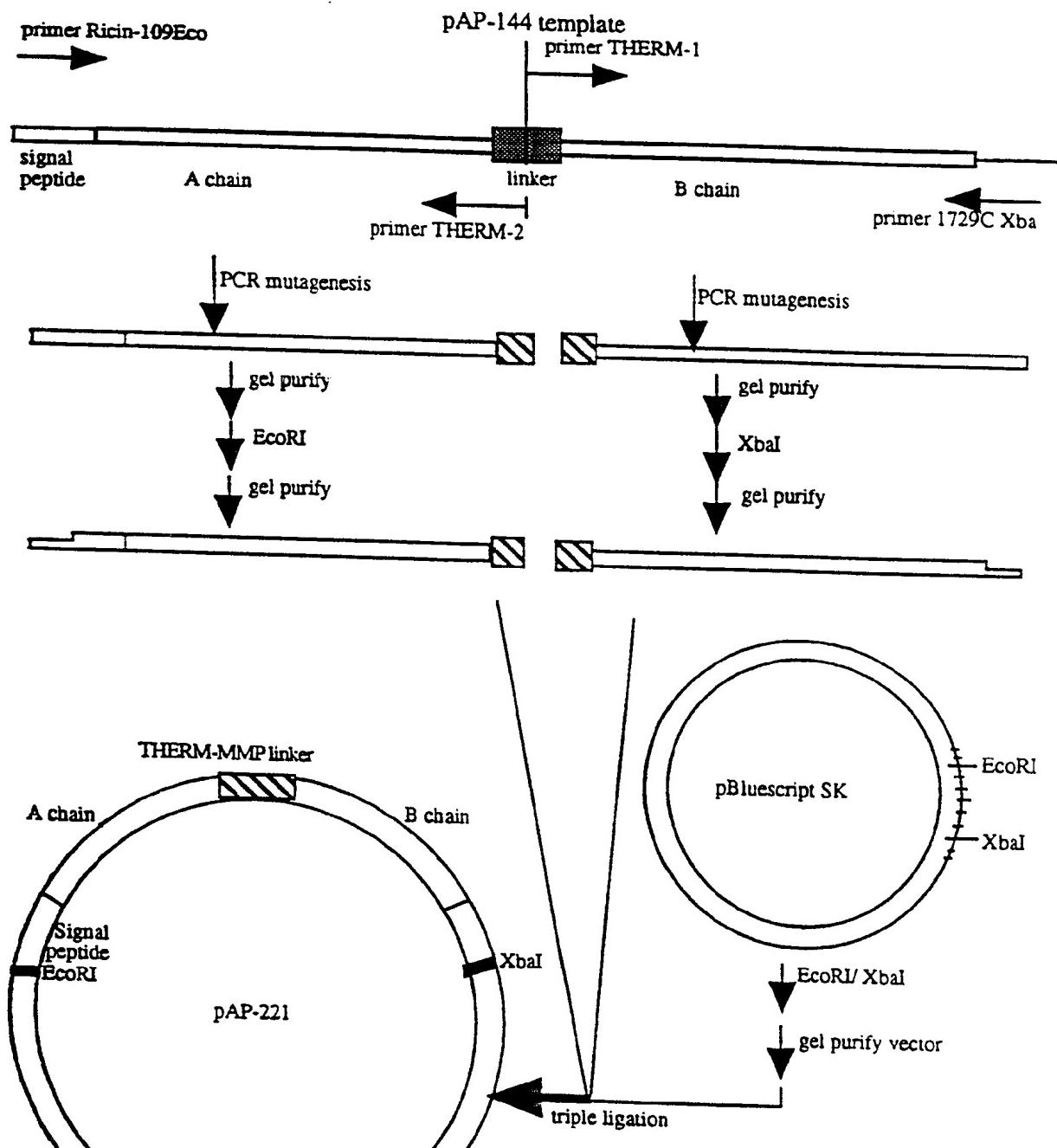
1 GAATTCATGAAACCGGGAGAATACTATTGTAAATGGATGTATGCAGT  
 CTTAAGTACTTTGCCCTCCTTATGATAACATTACACATACGTCA  
 51 GGCAACATGGCTTGGATCCACCTCAGGGTGGTCTTCACATTAG  
 CCGTTGTACCGAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAAC  
 101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCA  
 TCCTATTGTTGATAAGGGTTGTTATGGTTAATATTGAAATGGTGT  
 151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTCGCC  
 CGCCACGGTACACGTTGATGTGTTGAAATAGTCTCGACAAGCGCC  
 201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCAAGTGTGCCAA  
 AGCAAATTGTTGACCTCGACTACACTCTGACTATATGGTCACAACGGTT  
 251 ACAGAGTTGGTTGCCTATAAAACCAACGGTTATTTAGTTGAACCTCA  
 TGTCTAACCAACGGATATTGGTTGCCAAATAAAATCAACTTGAGAGT  
 301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA  
 TTAGTACGTCTCGAAAGACAATGTAATCGCGACTACAGTGGTTACGTAT  
 351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTCTTCATCCTGACA  
 ACACCAGCCGATGGCACGACCTTATCGGTATAAGAAAGTAGGACTGT  
 401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTCACTGATGTTCAAAAT  
 TAGTCCTCTACGTCTCGTAGTGGAGTAGAAAAGTGAACAGTTGTTA  
 451 CGATATACATTGCCCTTGGTGGTAATTATGATAGACTTGAACAACTTGC  
 GCTATATGTAAGCGAACCCACCATTAATAACTATCTGAACCTGTTGAACG  
 501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGGAGG  
 ACCATTAGACTCTCTTATAGCTAACCCCTTACCGAGGTGATCTCCCTCC  
 551 CTATCTAGCGCTTTATTACAGTACTGGTGGCACTCAGCTTCAACT  
 GATAGAGTCGCGAACATAATGTCATGACCACCGTGAGTCGAAGGTTGA  
 601 CTGGCTCGTCCCTTATAATTGCACTCCAAATGATTTCAAGCAGCAAG  
 GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTCGTT  
 651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA  
 TAAGGTTATATAACTCCCTCTTACCGTGCTCTTAATCCATGTTGCCCT  
 701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA  
 CTAGACGTGGTAGGATCGCATTATGTAACCTTATCAACCCCTCT  
 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT  
 GAAAGGTGACGTTAAGTCTCAGATTGGTCTCGAACGATCAGGTTA  
 801 TCAACTGCAAAGACGTAATGGTCCAAATTCACTGAGTACGATGTGAGTA  
 AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT  
 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCCACCTCCACCA  
 ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACGCGTGGAGGTGGT  
 901 TCGTCACAGTTCTCCGCAAGGAATTGCAGGGCAGCGAAATTAAATGC  
 AGCAGTGTCAAAGAGGCGTTCTAACGTCCCCTCGCTTAAATTACG

26/254

**FIGURE 5D (CONT'D)**

951 TGATTTGTATGGATCCTGAGCCCATAGTGCATCGTAGGTCGAAATG  
     ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
     CAGATACACAACATACTACCTACCTCTAAGGTGTTGCCCTTGCCTTAT  
 1051 CAGTTGTGCCATGCAAGCTAATACAGATGAAATCAGCTCTGGACTTT  
     GTCAACACCGGTACGTTAGATTATGCTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
     CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAAACTGCTGCA  
     CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC  
     TGACTACGGTGGCGACCCTTATACCCATTACCTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGAAACAGTGGTACCAACAC  
     GTCTAGATCAGATCAAAATCGTCGCTGAGTCCTGTACCATGGTGTG  
 1301 TTACAGTGCACCAACACATTATGCCGTTAGTCAGGTTGGCTTCTACT  
     AATGTCACGTTGGTTGTAATACGGCAATCAGTCCAACCGAAGGATGA  
 1351 AATAATACACAAACCTTTGTTACAACCATTGTTGGCTATATGGCTGTG  
     TTATTATGTGTTGGAAAACAATGTTGTAACAACCCGATAAACAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
     GAACGTTCGTTATCACCTGTTACACCTATCTCCTGACATCGTCACTTT  
 1451 AGGCTGAACAAACAGTGGCTTTATGCAGATGTTCAATACGTCCTCAG  
     TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
 1501 CAAACCGAGATAATTGCCCTACAAGTGATTCTAATATAACGGAAACAGT  
     GTTTGGCTCTATTAACGGAATGTTCACTAAGATTATGCCCTTGTCA  
 1551 TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
     ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCAGGGTGTACCTACA  
 1601 TCAAGAATGATGGAACCATTAAATTGTATAGTGGATTGGTGTAGAT  
     AGTTCTTACTACCTGGTAAATTAACATATCACCTAACCAACAACTCTA  
 1651 GTGAGGCATCGGATCCGAGCCTTAAACAAATCATTCTTACCCCTCTCCA  
     CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
 1701 TGGTGACCCAAACCAAATATGGTTACCAATTATTGTAGAGACAGATTACT  
     ACCACTGGGTTGGTTATACCAATGTAATAAAACTATCTGTCTAATGA  
 1751 CTCTTGCAGTGTGTGTCCTGCCATGAAAATAGATGGCTAAATAAAAAA  
     GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATT  
 1801 GGACATTGTAATTGTAACTGAAAGGACAGCAAGTTATATGAAATTC  
     CCTGTAACATTAAAACATTGACTTTCTGTGTTCAATATAGCTTAAGG  
 1851 TGCAG  
     ACGTC

27/254

**FIGURE 6A**

28 / 254

**FIGURE 6B**

## WT preprorocin linker

primer THERM-1

5' - AGGAATTGGCTTCTTTAGCTGATGTTGATG - 3'

TCTTTGCTATAAGGCCAGTGCTGCCAAATTAAAT

AGAAAACGAAATATTCCGGTCACACGGTTAAATTA

GCTGGTAGCAGTGTCAAACCTACACCTACTTCCCTACAC - 5'

3' -

primer THERM-2

PCR mutagenesis

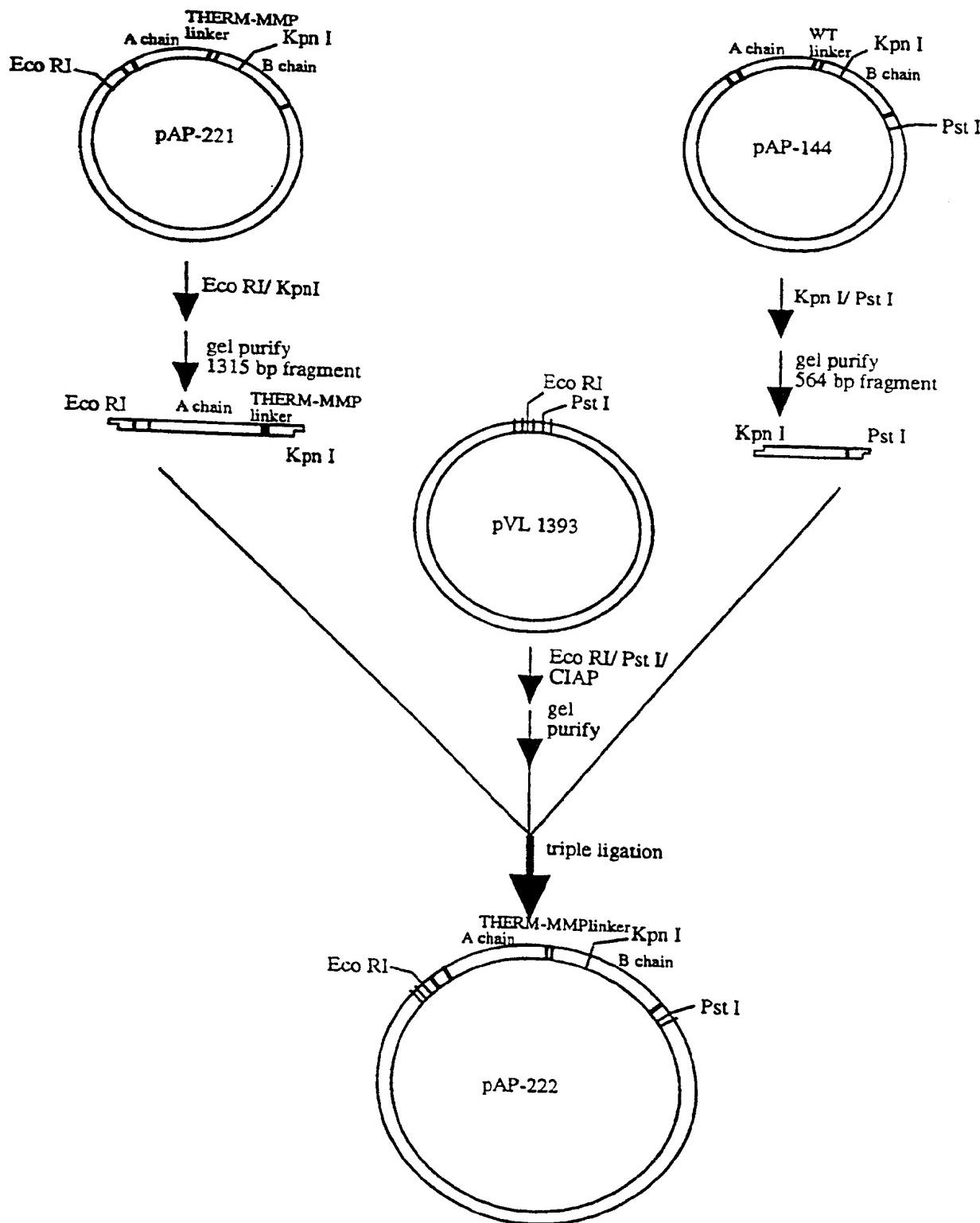
ligate with pBluescript SK

pAP221 linker  
(THERM-MMP variant)

New Cleavage Site

GATGTGGATGAAAGGATGTGAGGGAATTGCTCTTTTA  
CTAACACCTACTTTCCCTACACTCCCTAAACGAAGAAAAAT

29/254

FIGURE 6C

30/254

FIGURE 6D

10            20            30            40            50

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1  GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
   CTTAAGTACTTTGCCCTCCTTATGATAACATTACCTACATACGTCA

51  GGCAACATGGCTTGTGATCCACCTCAGGGTGGCTTCACATTAG
   CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC

101 AGGATAACAACATATTCCCCAACAAATACCCAAATTATAAACTTTACACAA
    TCCTATTGTTGATAAGGGTTGTTATGGTTAATATTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAAACCTTATCAGAGCTGTTCGCGG
    CGCCCACGGTGACACGTTGATGTGTTGAATAGTCTCGACAAGCGCC

201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATAACCAGTGTGCCAA
    AGCAAATTGTTGACCTCGACTACACTCTGACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACCTCTCA
    TGTCTCAACCAAACGGATATTGGTGCCTAAATAAACTCAACTTGAGAGT

301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
    TTAGTACGTCTCGAAAGACAATGTAATCGCACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTCTTCATCCTGACA
    ACACCAAGCCGATGGCACGACCTTATCGGTATAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTCACTGATGTTAAAAT
    TAGTCCTTCTACGTCTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTA

451 CGATATACATTGCCCTTGGTGGTAATTATGATAGACTTGAACAACTTGC
    GCTATATGTAAGCGAAACCACCATTAATACTATCTGAACCTGTTGAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG
    ACCATTAGACTCTCTTATAGCTAACCCCTTACCGAGTGTATCCTCC

551 CTATCTCAGCGCTTTATTACAGTACTGGTGGCACTCAGCTTCCAACCT
    GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGGTGA

601 CTGGCTCGTCCCTTATAATTGATCCAAATGATTTCAGAAGCAGCAAG
    GACCGAGCAAGGAAATATTAACGTAGGTTACTAAAGTCTTCGTCGGTTC

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
    TAAGGTTATATAACTCCCTCTTACCGTGCTCTTAATCCATGTTGGCCT

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA
    CTAGACGTGGTCTAGGATCGCATTATGTGAACCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT
    GAAAGGTGACGTTAAGTTCTCAGATTGGTCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTCCAAATTCAGTGTGTACGATGTGAGTA
    AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
    ATAATTAGGGATAGTATCGAGAGTACACATATCTACCGGTGGAGGTGGT

901 TCGTCACAGTTGATGTGGATGAAAGGGATGTGAGGGAAATTGCTTCTTT
    AGCAGTGTCAAACACACCTACTTCCCTACACTCCCTAAACGAAGAAA

951 TTTAGCTGATGTTGTATGGATCCTGAGCCCAGTGCCTACAGTGTAGGTC

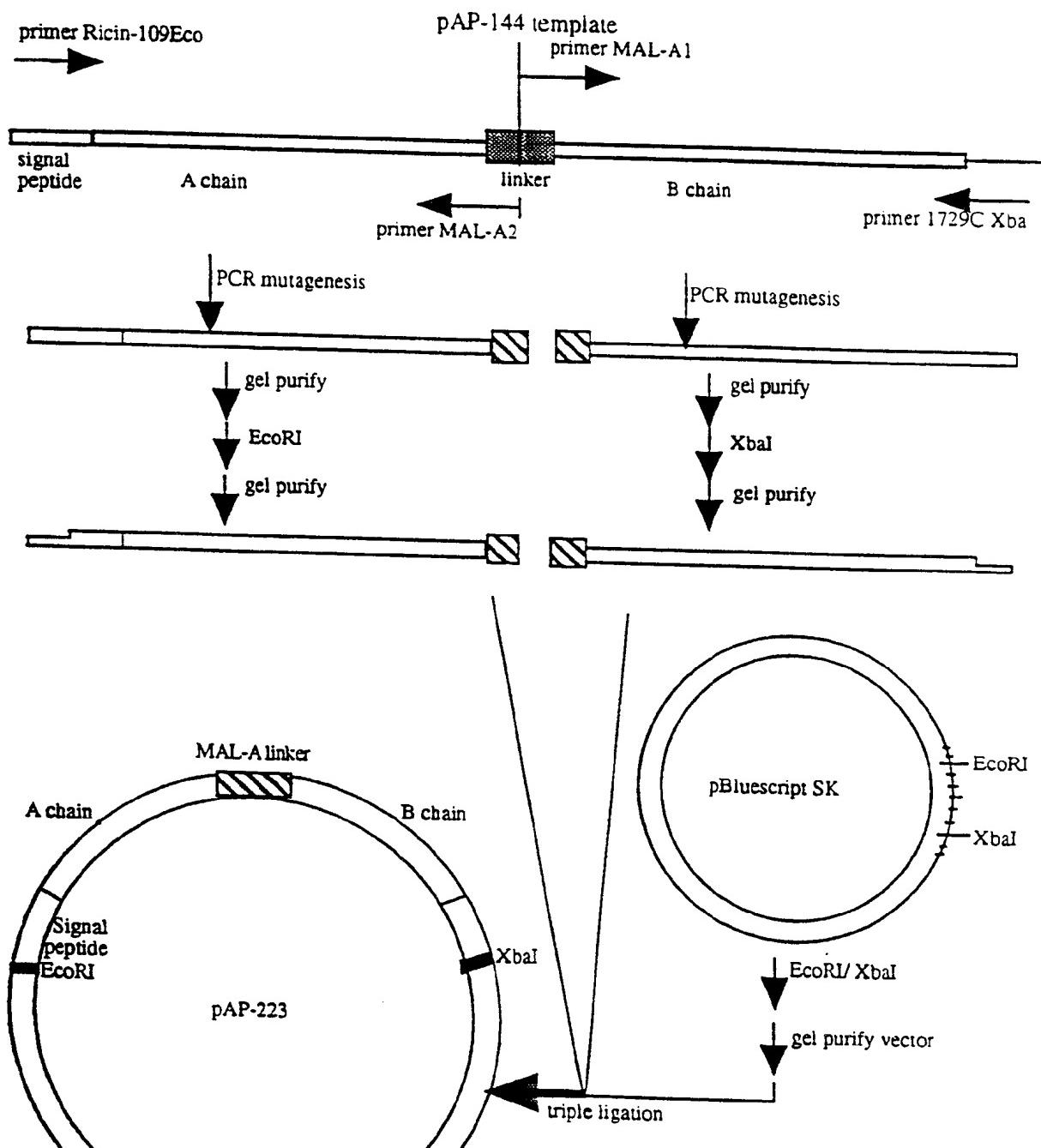
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31/254

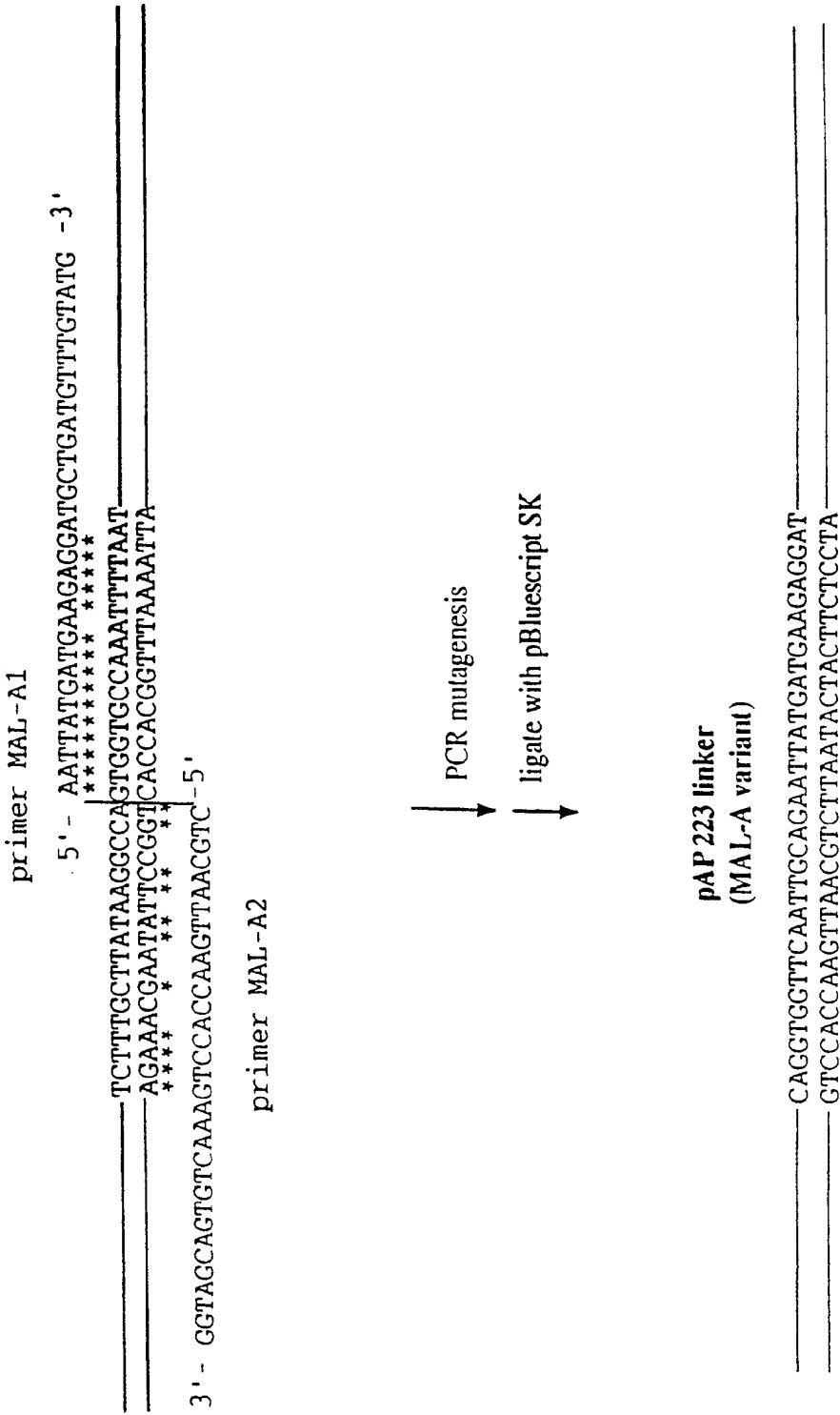
**FIGURE 6D (CONT'D)**

AAATCGACTACAAACATAACCTAGGACTCGGGTATCACGCATAGCATCCAG  
 1001 GAAATGGTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAAC  
 CTTTACCAAGATAACACAACATAATCCCTACCTCTAAGGTGTTGCCCTTG  
 1051 GCAATACAGTTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTG  
 CGTTATGTCAACACCCGGTACGTTACGATTATGTCTACGTTAGTCGAGAC  
 1101 GACTTTGAAAAGAGACAATACTATTGATCTAATGGAAGTGTAACTA  
 CTGAAACACTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGAT  
 1151 CTTACGGGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAAACT  
 GAATGCCCATGTCAGGCCCTCAGATAACACTACTAGATAACTAACGTTATGA  
 1201 GCTGCAACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCAT  
 CGACGTTGACTACGGTGGCGACCCTTATACCCATTACCTTGGTAGTA  
 1251 AAATCCCAGATCTAGTCTAGTTTACAGCAGCAGATCAGGGAACAGTGGTA  
 TTTAGGGTCTAGATCAGATCAAATCGTCGCTGTAGTCCTTGTCAACCAT  
 1301 CCACACTTACAGTGCAAACCAACATTATGCCGTTAGTCAAGGTTGGCTT  
 GGTGTGAATGTCACGTTGGTTGTAACACGGCAATCAGTTCCAACCGAA  
 1351 CCTACTAATAACACAAACCTTTGTTACAACCAATTGTTGGCTATATGG  
 GGATGATTATTATGTGTTGGAAAACAATGTTGGTAACACCCGATATACC  
 1401 TCTGTGCTTGCAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCA  
 AGACACGAACGTTGTTATCACCTGTCATACTATCTCTGACATCGT  
 1451 GTGAAAAGGCTGAACAAACAGTGGGCTTTATGCAGATGGTCAATACGT  
 CACTTTCCGACTTGTGTCACCCGAGAAATACTACGTTACCAAGTTATGCA  
 1501 CCTCAGCAAACCGAGATAATTGCCCTACAAGTGTATTCTAATATACGGGA  
 GGAGTCGTTGGCTCTATTACGGAATGTTCACTAAGATTATATGCCCT  
 1551 AACAGTTGTTAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGAT  
 TTGTCAACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTA  
 1601 GGATGTTCAAGAATGATGGAACCAATTAAATTGTAATGTGGATTGGTG  
 CCTACAAGTTCTACTACCTTGGTAAATTAAACATATCACCTAACAC  
 1651 TTAGATGTGAGGCAGTCGGATCCGAGCCTTAAACAAATCATTCTTACCC  
 AATCTACACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGG  
 1701 TCTCCATGGTACCCAAACCAATATGGTACCAATTATTTGATAGACAG  
 AGAGGTACCACTGGGTTGGTTATACCAATGGTAATAAAACTATCTGTC  
 1751 ATTACTCTTGCAGTGTGTTGTCCTGCCATGAAAATAGATGGCTTAAA  
 TAATGAGAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATT  
 1801 TAAAAAGGACATTGTAATTGTAACGTAAAGGACAGCAAGTTATATCG  
 ATTTTCTGTAACATTAAAACATTGACTTCTGTCGTTCAATATAGC  
 1851 AATTCCCTGCAG  
 TTAAGGACGTC

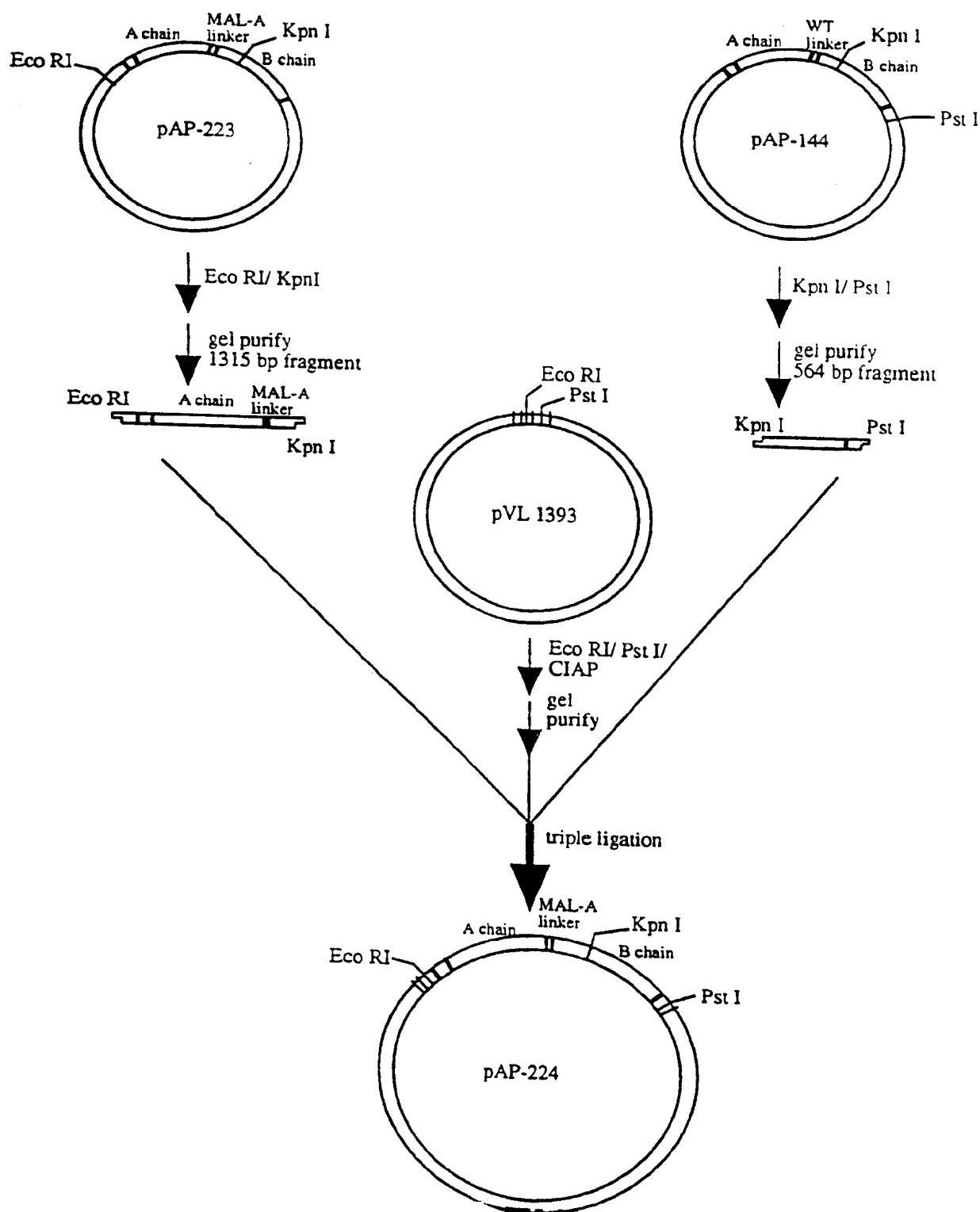
32/254

**FIGURE 7A**

33/254

**FIGURE 7B****WT preprorcin linker**

34/254

**FIGURE 7C**

35 / 254

## FIGURE 7D

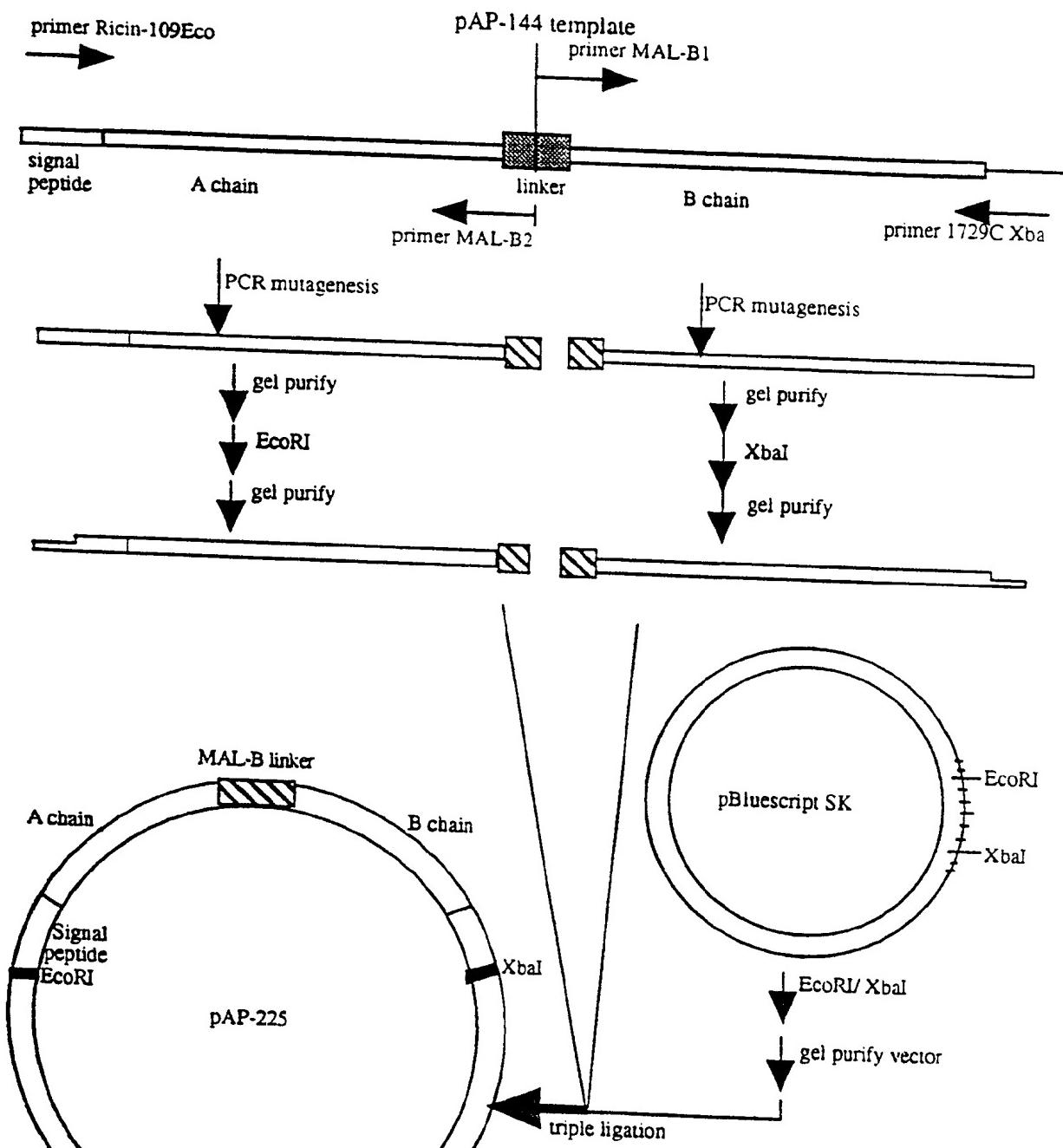
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1 GAATTCATGAAACCGGGAGGAATACTATTGTAATATGGATGTATGCAGT  
CTTAAGTACTTTGCCCTCCTTATGATAACATTACACATACATACGTCA  
51 GGCAACATGGCTTGTGGATCCACCTCAGGGTGGCTTCACATTAG  
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAACTC  
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAATTTACCA  
TCCTATTGTTGTATAAGGGGTTGTTATGGGTTAATATTGAAATGGTGT  
151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTCGCCG  
CGCCCCACGGTGACACGTTGATGTGTTGAAATAGTCTGACAAGCGCC  
201 TCGTTTAACAACACTGGAGCTGATGTGAGACATGATATACCAGTGTGCCAA  
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT  
251 ACAGAGTTGGTTGCCATAAACCAACGGTTATTTAGTTGAACTCTCA  
TGTCTCAACCAACGGATATTGTTGCCAAATAAACTCAACTTGAGAGT  
301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA  
TTAGTACGTCTCGAAAGACAATGTAATCGCACCTACAGTGGTTACGTAT  
351 TGTGGTCGGTACCGTGCTGGAAATAGCGCATATTCCTTCATCCTGACA  
ACACCAGCCGATGGCACGACCTTATCGGTATAAAGAAAGTAGGACTGT  
401 ATCAGGAAGATGCAGAACGAAATCACTCATCTTCACTGATGTTCAAAAT  
TAGTCCTTCTACGTCTCGTTAGTAGAAAAGTAGACTACAAGTTTA  
451 CGATATACATTGGCTTGGTGGTAATTATGATAGACTGAAACAACCTGC  
GCTATATGTAAGCGAACCAACCATTAATACTATCTGAACTTGTGAACG  
501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG  
ACCATTAGACTCTCTTTATAGCTCAACCCTTACAGGTGATCTCCTCC  
551 CTATCTCAGCGCTTATTATTACAGTACTGGTGGCACTCAGCTTCCA  
GATAGAGTCGGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA  
601 CTGGCTCGTCCCTTATAATTGCAATCCAATGATTCAGAACGCAGCAAG  
GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTCTTC  
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA  
TAAGGTTATATAACTCCCTCTTACCGGTGCTCTTAAATCCATGTTGGCCT  
701 GATCTGCACCAAGATCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA  
CTAGACGTGGTCTAGGATCGCATTATGTGAACACTCTTATCAACCCCCCTCT  
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT  
GAAAGGTGACGTTAAGTCTCAGATTGGTCTCGGAAACGATCAGGTTA  
801 TCAACTGCAAAGACGTAATGGTCCAAATTCACTGAGTACGATGAGTA  
AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT  
851 TATTAATCCCTATCATAGCTCTAGGTGTTAGATGCGCACCTCCACCA  
ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACGGTGGAGGTGGT  
901 TCGTCACAGTTTCAGGGTTCAATTGCAAGAATTATGATGAAAGAGGATGC  
AGCAGTGTCAAAGTCCACCAAGTTAACGTCCTTAATACTACTTCTCCTACG

36/254

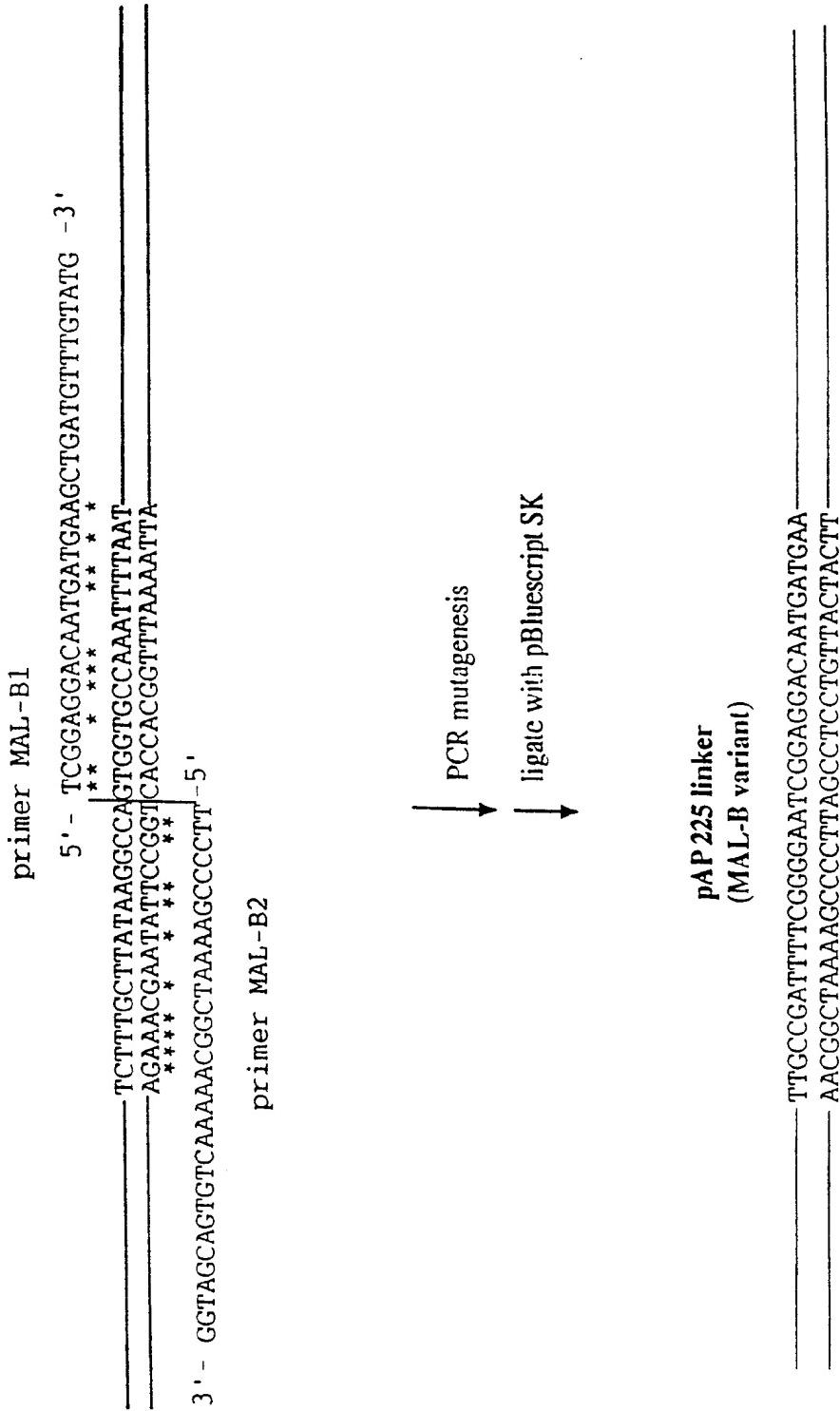
**FIGURE 7D (CONT'D)**

951 TGATGTTGTATGGATCCTGAGCCCATAGTGCCTATCGTAGGTCGAAATG  
 ACTACAAACATACACTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
 CAGATAACACAACATACAATCCCTACCTCTAAGGTGTTGCCCTTGCGTTAT  
 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTAGATTATGTCTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGGAGTCATGTGATGATCTATGATTGCAAAACTGCTGCA  
 CCATGTCAGGCCCTCAGATAACACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGATAATGGAACCACATCATAAATCC  
 TGACTACGGTGGCGACCGTTTACCTTACCTTACCTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
 GTCTAGATCAGATAACCGTCTGCTGTTAGTCCTTGTCACCATGGTGTG  
 1301 TTACAGTGCAAACCAACATTATGCCGTTAGTCAGGTTGGCTTCAACT  
 AATGTCACGTTGGTTGAAATACGGCAATCAGTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG  
 TTATTATGTGTTGGAAAACAATGTGGTAACACCCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGAGCTGTAGCAGTGAAA  
 GAACGTTGTTTACCTGTTACCTATCTCCTGACATCGTCACTTT  
 1451 AGGCTGAACAAACAGTGGCTTTATGCAGATGGTCAATAACGTCTCAG  
 TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTTACAAGTGATTCTAATATAACGGAAACAGT  
 GTTTGGCTCTTAAACGGAAATGTTCACTAAAGATTATATGCCCTTGTCA  
 1551 TGTTAACGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
 ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGGTGTACCTACA  
 1601 TCAAGAACGATGGAAACCAATTAAATTGTTAGTGTAGGGATTGGTGTAGAT  
 AGTTCTTACTACCTGGTAAATTAACATATCACCTAACCAACAACTCTA  
 1651 GTGAGGCGATGGATCGGAGCCTTAAACAAATCATTCTTACCTCTCCA  
 CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
 1701 TGGTGACCCAAACCAAAATATGGTACCAATTATTTGATAGACAGATTACT  
 ACCACTGGGTTGGTTACCAATGGTAATAAAACTATCTGTCTAAATGA  
 1751 CTCTTGCAGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAAA  
 GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATTTTT  
 1801 GGACATTGTAATTTGTAACGTGAAAGGACAGCAAGTTATATCGAATTCC  
 CCTGTAACATTAAAACATTGACTTCCCTGTTCAATATAGCTTAAGG  
 1851 TGCAG  
 ACGTC

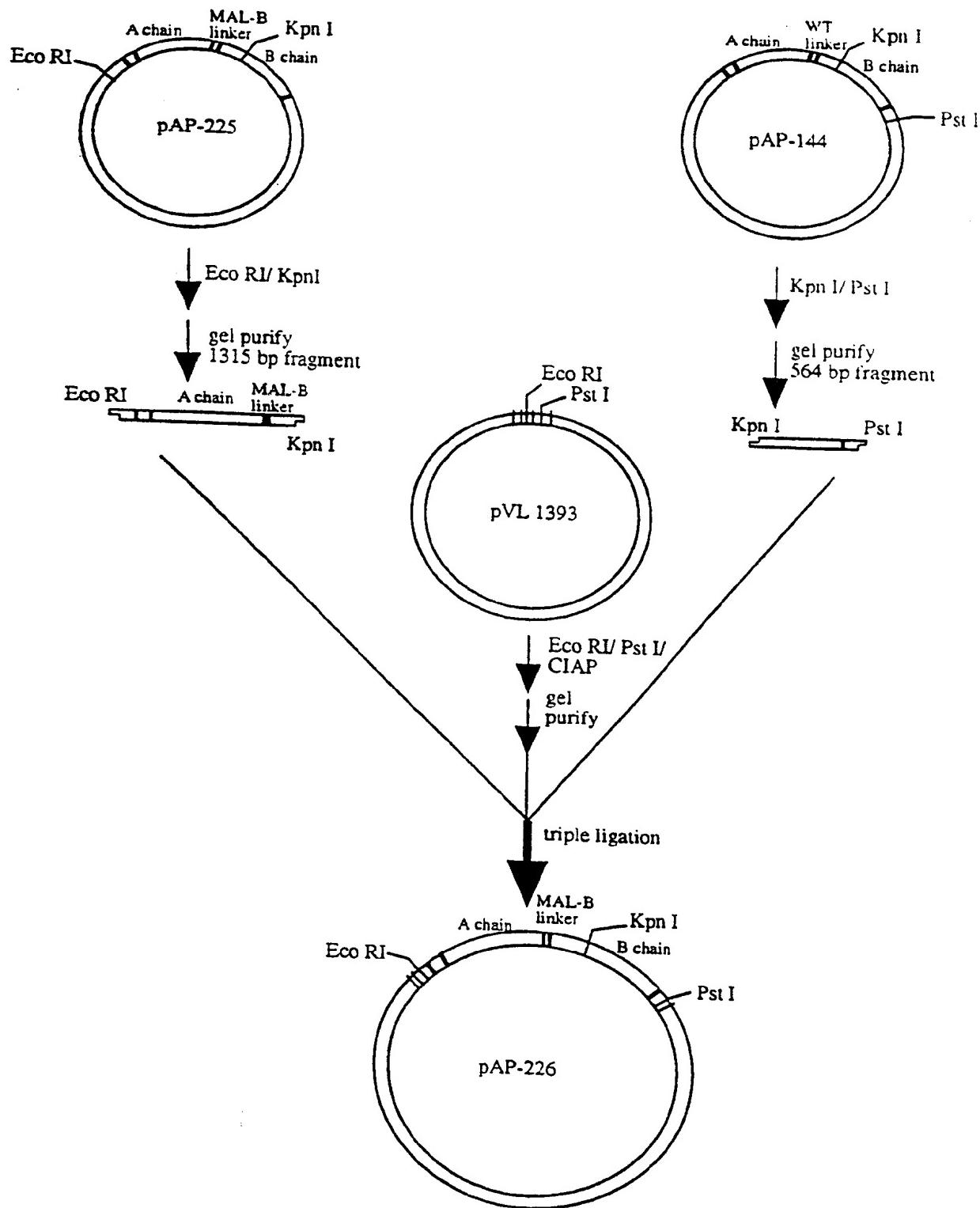
37/254

FIGURE 8A

38 / 254

**FIGURE 8B****WT preprorocin linker**

39/254

**FIGURE 8C**

40/254

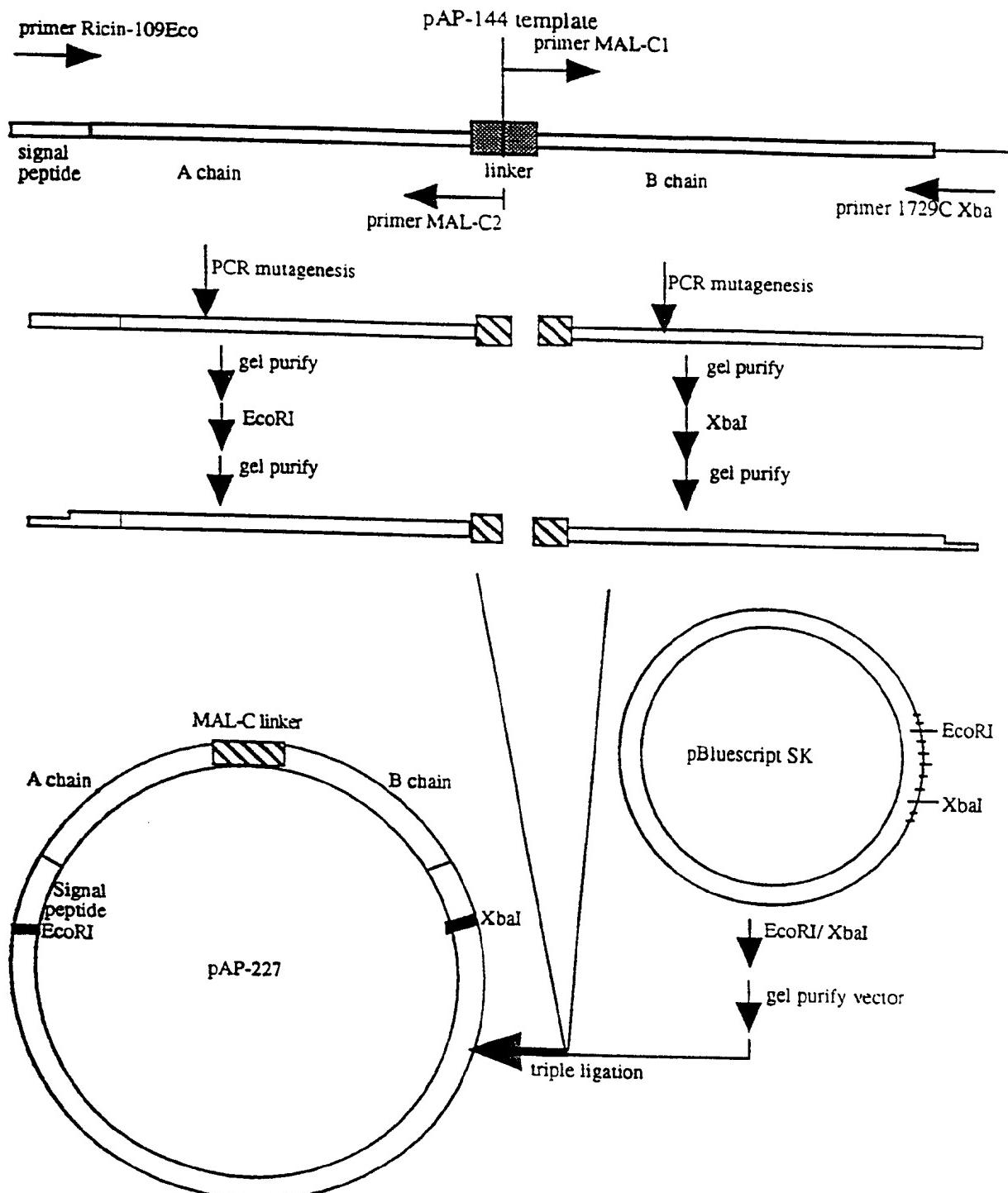
FIGURE 8D

1	10	20	30	40	50
GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT					
CTTAAGTACTTTGCCCTCCTTATGATAACATTATACTACATACGTCA					
51 GGCAACATGGCTTGTGATCCACCTCAGGGGGCTTCACATTAG					
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC					
101 AGGATAACAAACATATTCCCCAAACAATACCCAATTATAAACCTTACCA					
TCCTATTGTTGTATAAGGGGTTGTTATGGGTTAATATTGAAATGGTGT					
151 GCGGGTGCCTACTGTGCAAAGCTACACAAACCTTATCAGAGCTGTCGCGG					
CGCCCCACGGTGACACGTTGATGTGTTGAAATAGTCTCGACAAGCGCC					
201 TCGTTAACAAACTGGAGCTGATGTGAGACATGATATAACCAAGTGTGCAA					
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTACAAACGGTT					
251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACTCTCA					
TGTCTCAACCAACGGATATTGGTGCCTAAATAACACTGAGAGT					
301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA					
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT					
351 TGTGGTCGGCTACCGTGTGAAATAGCGCATATTCTTCATCCTGACA					
ACACCAAGCCGATGGCACGACTTATCGGTATAAAAGAAAGTAGGACTGT					
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTCACTGATGTTCAAAT					
TAGTCCTTCTACGTCTCGTAGTGAGTAGAAAAGTGAACACTAAGTTTA					
451 CGATATACATTGCCCTTGGTAATTATGATAGACTTGAACAACCTGC					
GCTATATGTAAGCGGAAACCACCAATTAAACTATCTGAACCTGTTGAACG					
501 TGGTAATCTGAGAGAAAAATCGAGTTGGAAATGGTCCACTAGAGGAGG					
ACCATTAGACTCTTTATAGCTAACCTTACAGGTGATCTCCTCC					
551 CTATCTAGCGCTTATTATACAGTACTGGTGGACTCAGCTTCAA					
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA					
601 CTGGCTCGTCCTTATAATTGATCAAATGATTTCAGAAGCAGCAAG					
GACCGAGCAAGGAAATATTAAACGTAGTTACTAAAGTCTCGTCGTT					
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA					
TAAGGTTATATAACTCCCTTTACGCGTGTCTTAATCCATGTTGGCCT					
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA					
CTAGACGTGGTCTAGGATCGCATTATGTAACCTTATCAACCCCTCT					
751 CTTTCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCAAAT					
GAAAGGTGACGTTAAGTCTCAGATTGTTCTCGGAAACGATCAGGTTA					
801 TCAACTGCAAAGACGTAATGGTCAAATTCACTGAGATGTGAGTA					
AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT					
851 TATTAATCCCTATCATAGCTCATGGTGTATAGATGCGCACCTCCACCA					
ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACGCGTGGAGGTGGT					
901 TCGTCACAGTTTGCCGATTTGGGGAAATGGAGGACAATGATGAAGC					
AGCAGTGTCAAAACGGCTAAAGCCCTTAGCCTCCTGTTACTACTTCG					

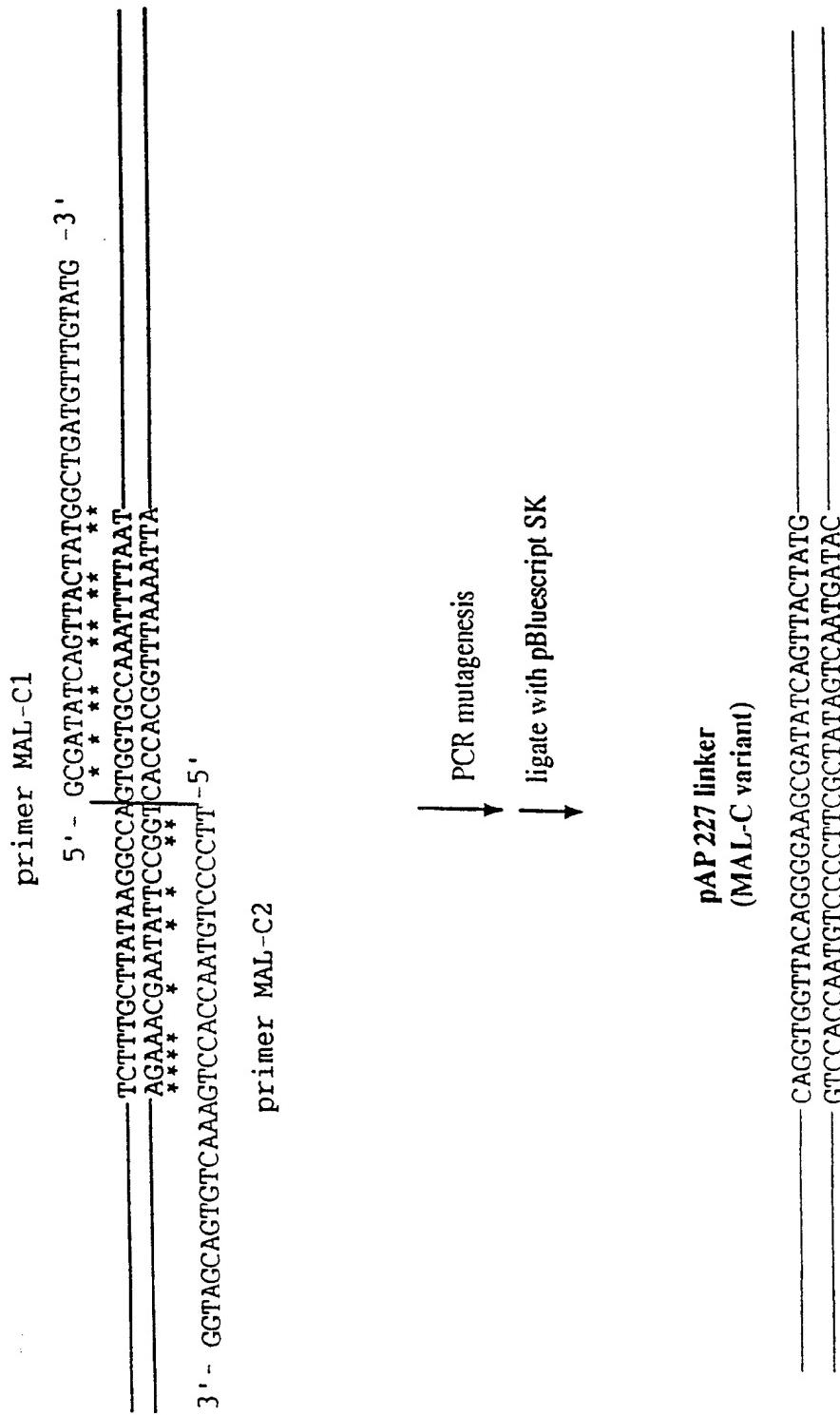
**FIGURE 8D (CONT'D)**

951 TGATTTGTATGGATCCTGAGCCCATACTGCCTCGTAGGTCGAAATG  
 ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
 CAGATAACACAACATACTACCTACCTCTAAGGTGTTGCCTTGCGTTAT  
 1051 CAGTTGTGCCATGCAACTCTAATAACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTCAGATTATGTCACGTTAGTCAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAAAACTGCTGCA  
 CCATGTCAGGCCCTCAGATACTACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCACATCATAAATCC  
 TGACTACGGTGGCGACCGTTATACCTATTACCTTGGTAGTATTTAGG  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
 GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTGTCACCATGGTGTG  
 1301 TTACAGTCAAACCAACATTATGCCGTTAGTCAGGTTGGCTTCTACT  
 AATGTCACGTTGGTTGTAACACGGCAATCAGTTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG  
 TTATTATGTTGGAAAACAATGTTGTTACAACACCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
 GAACGTTCGTTATCACCTGTTACACCTATCTCCTGACATCGTCACTTT  
 1451 AGGCTGAACAAACAGTGGGCTCTTATGCAGATGGTCAATACGTCCTCAG  
 TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTTACAAGTGATTCTAATAACGGAAACAGT  
 GTTTGGCTCTATTACGGAATGTTACTAAGATTATATGCCCTTGTC  
 1551 TGTTAAGATCCTCTTGCGGCCCTGCATCCTCTGGCCAACGATGGATGT  
 ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA  
 1601 TCAAGAATGATGGAACCATTAAATTGTTAGTGGATTGGTTAGAT  
 AGTTCTTACTACCTTGGTAAATTAACATATCACCTAACCAACAACTCTA  
 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTACCCCTCCA  
 CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
 1701 TGGTGACCCAAACCAAATGGTTACCAATTATTGATAGACAGATTACT  
 ACCACTGGGTTGGTTATACCAATGTAATAAAACTATCTGCTAATGA  
 1751 CTCTTGCAGTGTGTCCTGCCATGAAAATAGATGGCTAAATAAAAAA  
 GAGAACGTCACACACACAGGACCGTACTTTATCTACCGAATTATT  
 1801 GGACATTGTAATTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC  
 CCTGTAACATTTAAACATTGACTTCCCTGTCGTTCAATATAGCTTAAGG  
 1851 TCCAG  
 ACGTC

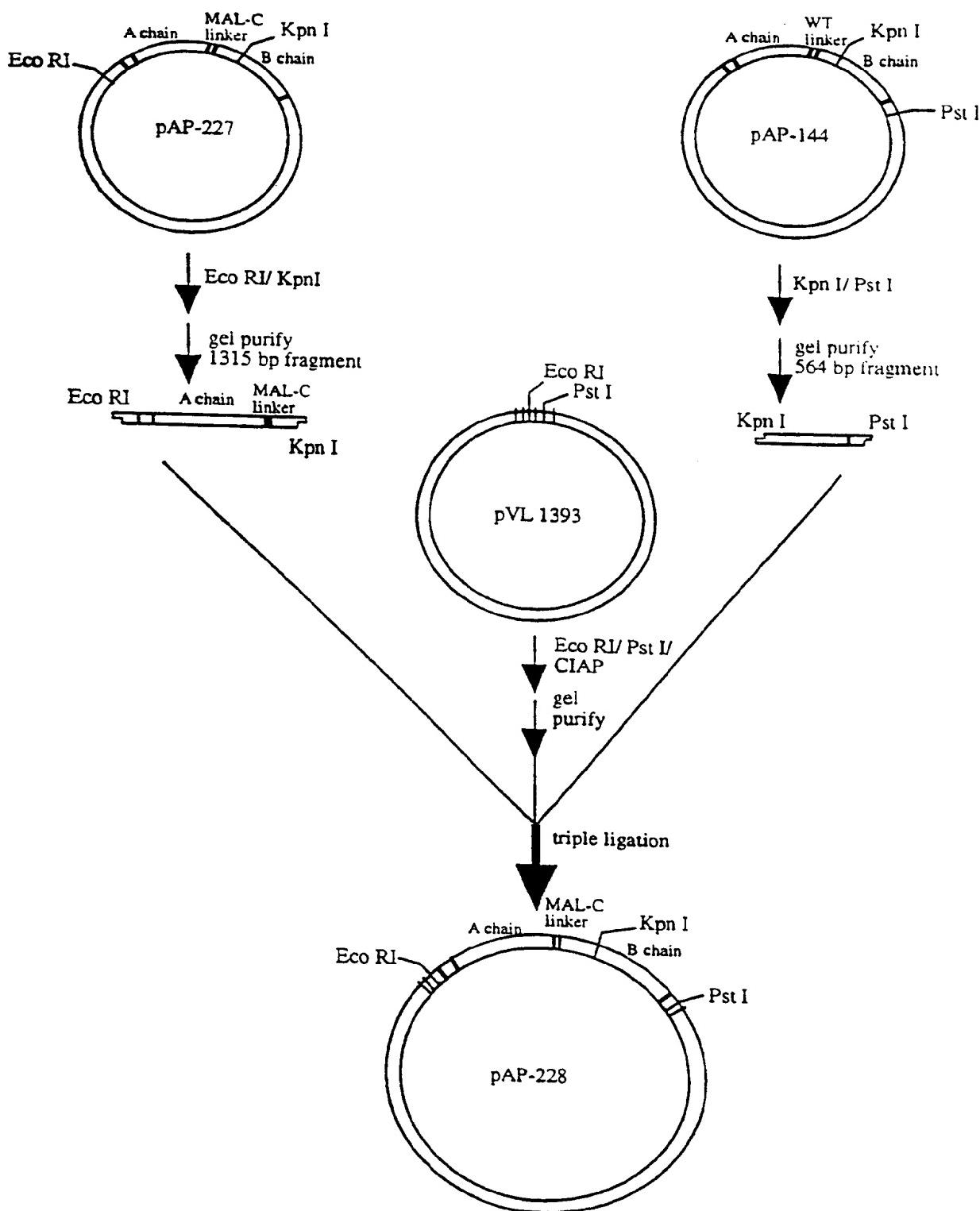
42/254

**FIGURE 9A**

43/254

**FIGURE 9B****WT preprorcin linker**

44/254

FIGURE 9C

45/254

**FIGURE 9D**

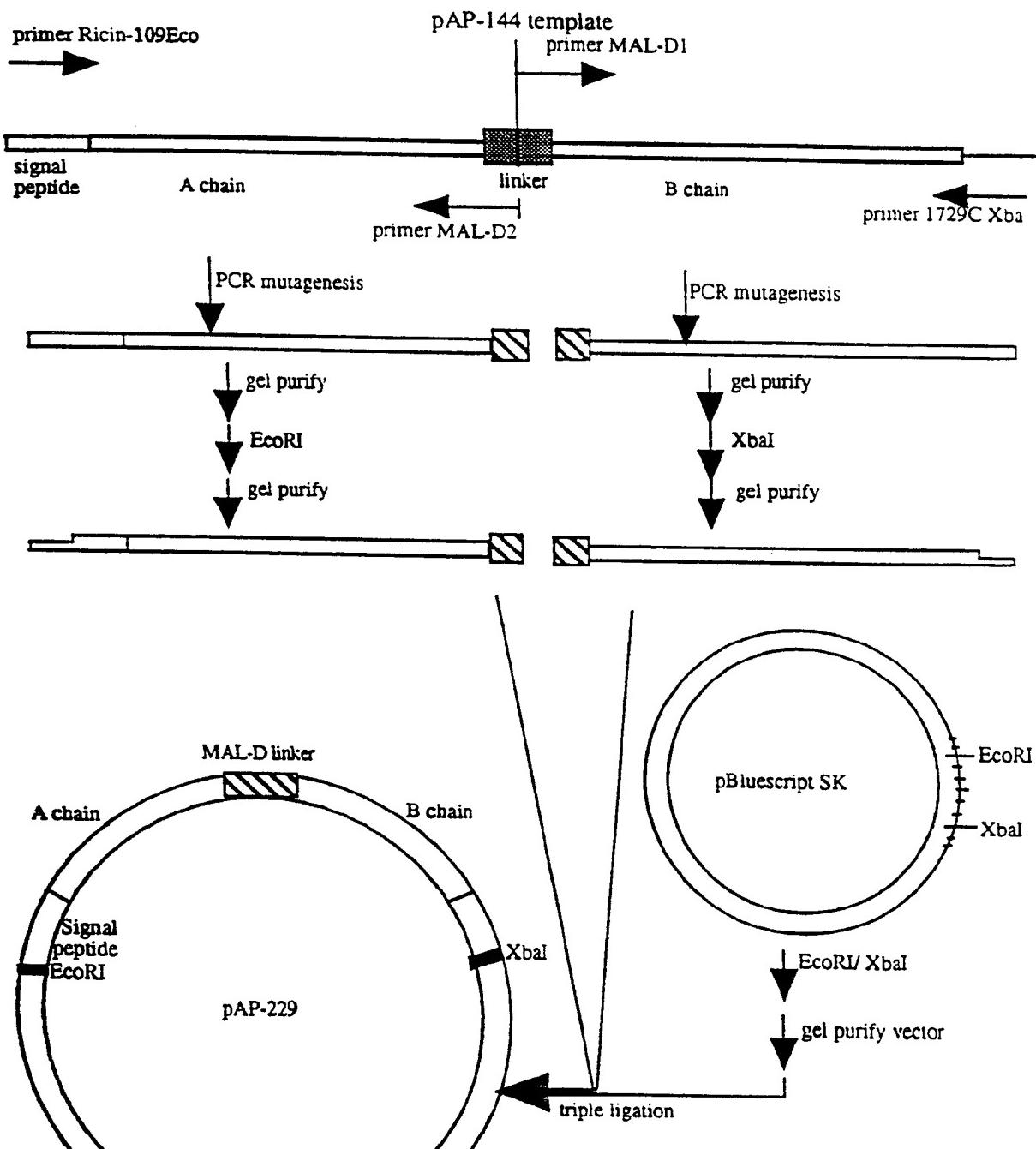
10	20	30	40	50
1 GAATTCATGAAACCGGGAGGAATACTATTGTAATATGGATGTATGCAGT				
CTTAAGTACTTTGCCCTCCTTATGATAACATTATACTACATACGTCA				
51 GGCAACATGGCTTGTGATCCACCTCAGGGGGTCTTCACATTAG				
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC				
101 AGGATAACAACATATTCCCCAACAAATACCCAATTATAAACTTACCA				
TCCTATTGTTGATAAGGGGTTGTTATGGGTTAATATTGAAATGGTGT				
151 GCGGGTGCCACTGTGCAAAGCTACACAAAACCTTATCAGAGCTGTTCGGG				
CGCCCACGGTGAC-CGTTCGATGTGTTGAAATAGTCTCGACAAGCGCC				
201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCAAGTGTGCAA				
AGCAAATTGTTGACCTCGACTACACTCTGACTATATGGTCACAACGGTT				
251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACTCTCA				
TGTCTCAACCAACGGATATTGGTGCCTAACAAATCAACTTGAGAGT				
301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA				
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT				
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTCTTCATCCTGACA				
ACACCAGCCGATGGCACGACTTATCGGTATAAAGAAAGTAGGACTGT				
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTCACTGATGTTAAAAT				
TAGTCCTTCTACGTCTCGTTAGTAGAAAGTAGACTACAAGTTTA				
451 CGATATACATTGCCCTTGGGTAATTATGATAGACTTGAACAACTTGC				
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACCTGTTGAACG				
501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG				
ACCATTAGACTCTTTATAGCTAACCCCTTACCGAGGTGATCTCCTCC				
551 CTATCTCAGCGTTATTACAGTACTGGTGGCACTCAGCTTCAACT				
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA				
601 CTGGCTCGTTCTTATAATTGATCCAAATGATTCAGAAGCAGCAAG				
GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTCGTT				
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAAATTAGGTACAACCGGA				
TAAGGTTATATAACTCCCTTTACCGGTGCTTTAATCCATGTTGGCCT				
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAAATAGTTGGGGAGA				
CTAGACGTGGTCTAGGATCGCATTAAATGTAACCTTATCAACCCCTCT				
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCAA				
GAAAGGTGACGTTAAGTCTCAGATTGGTCTCGGAAACGATCAGGTTA				
801 TCAACTGCAAAGACGTAATGGTCAAATTCACTGTTGACGATGTGAGTA				
AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT				
851 TATTAATCCCTATCATAGCTCTCATGGTGTAGATGCGCACCTCCACCA				
ATAATTAGGGATAGTATCGAGAGTACCCACATATCTACCGTGAGGTGGT				
901 TCGTCACAGTTTCAGGTGGTACAGGGGAAGCGATATCAGTTACTATGGC				
AGCAGTGTCAAAGTCCACCAATGTCCTCGCTATAGTCATGATAACCG				

46/254

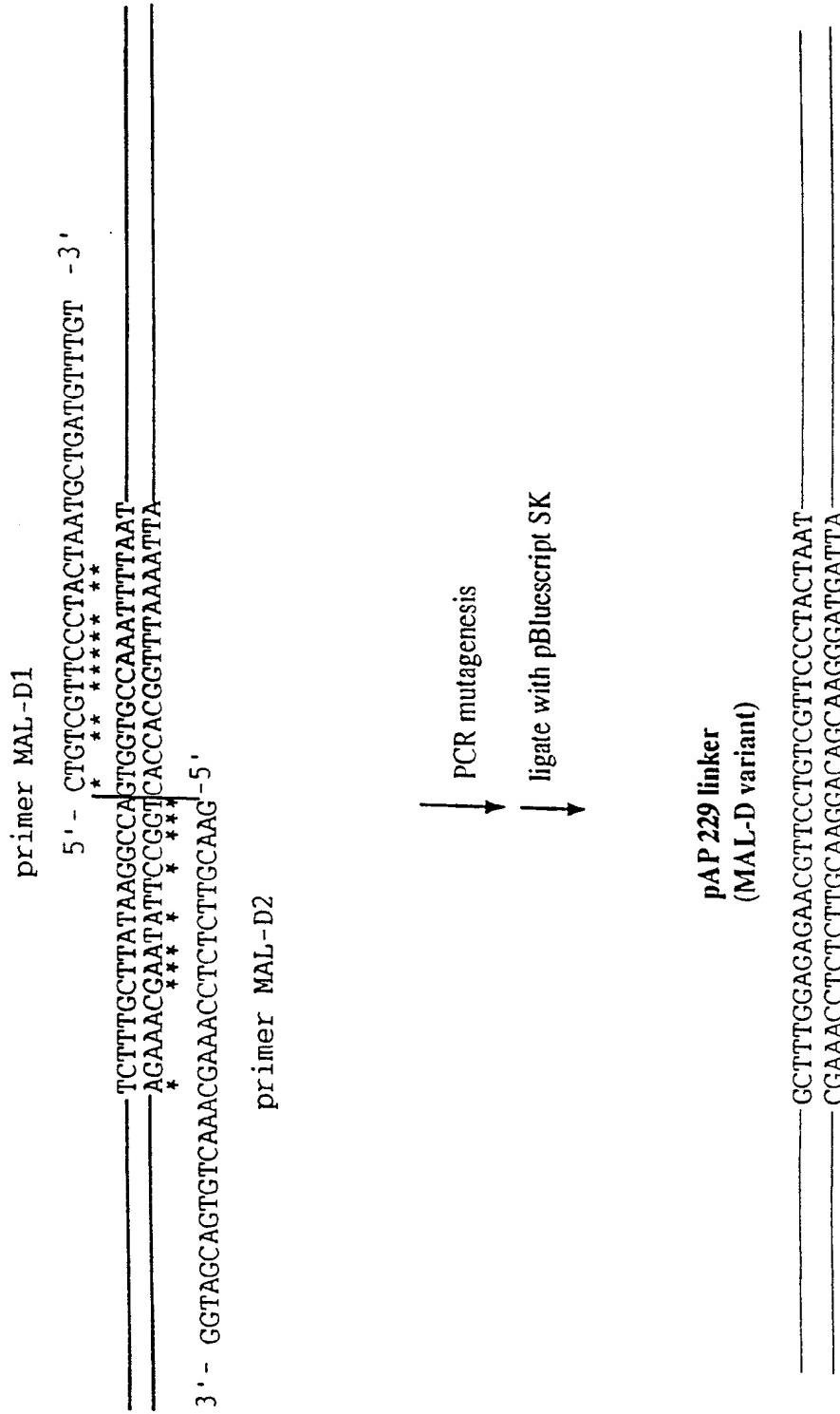
**FIGURE 9D (CONT'D)**

951 TGATTTGTATGGATCCTGAGCCCATAGTCGTATCGTAGGTCGAAATG  
 ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
 CAGATACACAACATAACATCCCTACCTTCTAAGGTGTTGCCTTGCCTTAC  
 1051 CAGTTGTGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCAGGTACGTTACGATTATGTCAGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAAACTGCTGCA  
 CCATGTCAGGCCCTCAGATACACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC  
 TGACTACGGTGGCGACCGTTATACCTTATTACCTTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
 GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTTGTACCATGGTGTG  
 1301 TTACAGTGCAAACCAACATTATGCCGTTAGTCAGGTTGGCTTCTACT  
 AATGTCACGTTGGTTAAATACGGCAATCAGTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
 TTATTATGTTGGAAAACAATGTTGGTAACAACCCGATATACCAAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
 GAACGTTGTTATCACCTGTTACACCTATCTCTGACATCGTCACTT  
 1451 AGGCTGAACAAACAGTGGCTTTATGCAGATGGTTCAATACGTCCTCAG  
 TCCGACTTGGTGTACCCGAGAAAACAGTCTACCAAGTTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGATTCTAATATACGGAAACAGT  
 GTTTGGCTCTATTAACGGAATGTTACTAAGATTATATGCCCTTGTCA  
 1551 TGTAAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
 ACAATTCTAGGAGAGAACACCCGGACGTAGGAGACCGGTTGCTACCTACA  
 1601 TCAAGAATGATGGAACCATTAAATTGTATAGTGGATTGGTGTAGAT  
 AGTTCTACTACCTGGTAAATTAAACATATCACCTAACCAACAACTCTA  
 1651 GTGAGGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTACCCCTCTCCA  
 CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
 1701 TGGTGACCCAAACCAAATATGGTTACCAATTATTTGATAGACAGATTACT  
 ACCACTGGGTTGGTTATACCAATGTAATAAAACTATCTGTCTAATGA  
 1751 CTCTTGCAGTGTGTGTCCTGCCATGAAAATAGATGGCTAAATAAAAAA  
 GAGAACGTCACACACACAGGACGGTACTTTATCACCGAATTATTTT  
 1801 GGACATTGTAATTGGTAACTGAAAGGACAGCAAGTTATATCGAATTCC  
 CCTGTAACATTAAAACATTGACTTCCCTGTCGTTCAATATAGCTTAAGG  
 1851 TGCAG  
       ACGTC

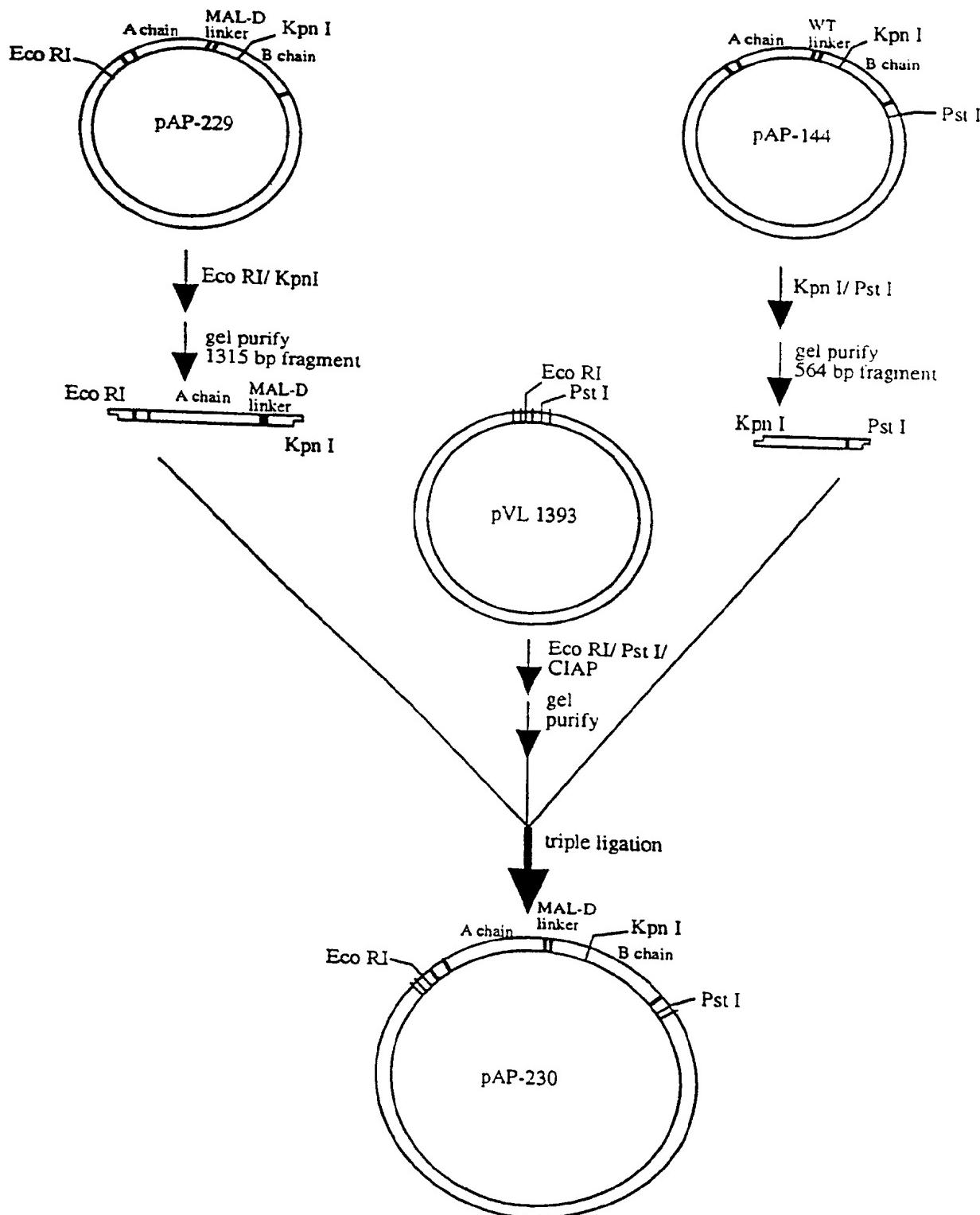
47/254

FIGURE 10A

48 / 254

**FIGURE 10B****WT preprorcin linker**

49/254

FIGURE 10C

50/254

FIGURE 10D

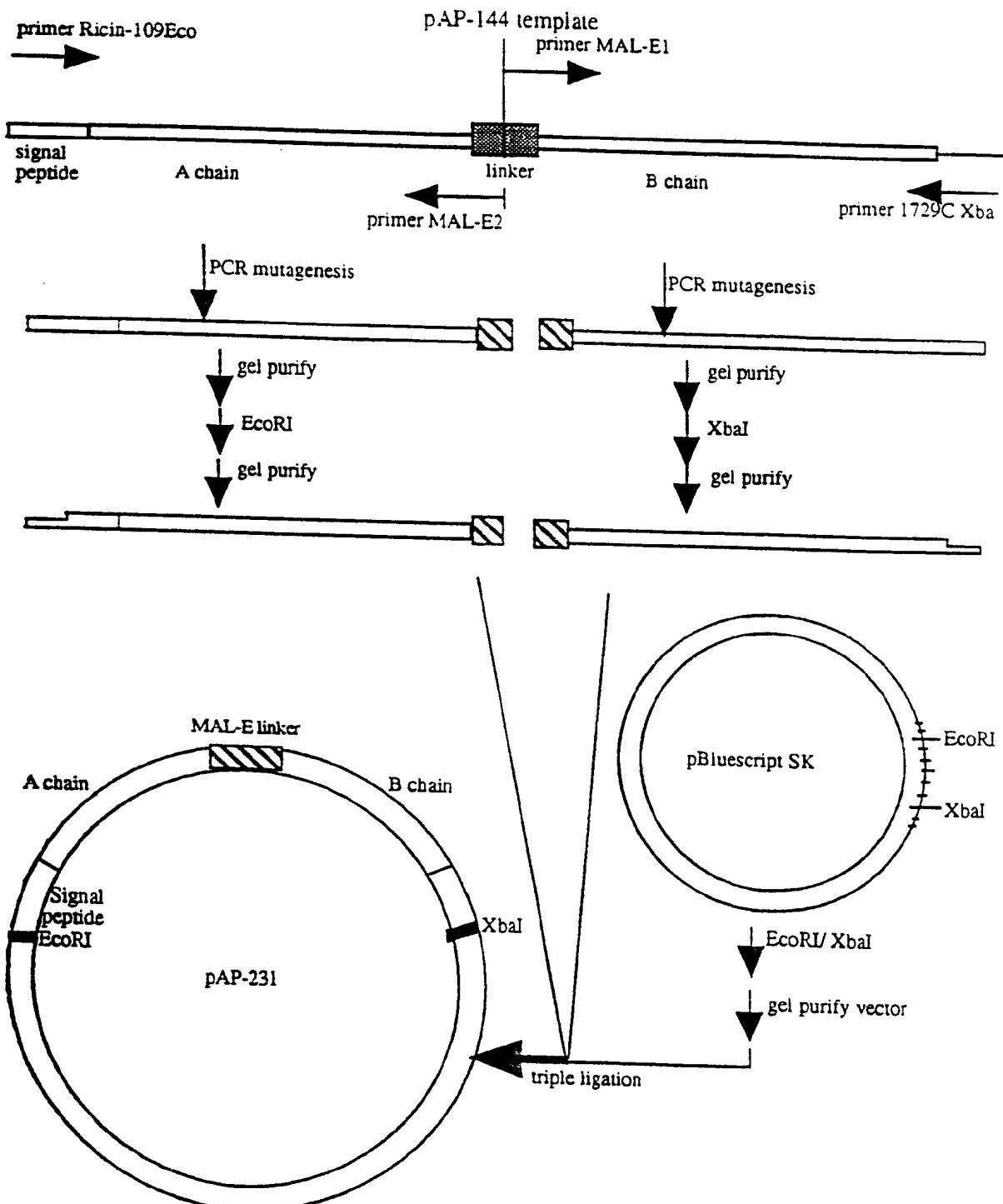
10	20	30	40	50
1 GAATT	CATGAA	ACCGGGAGGA	AATACTATTG	TATGGATGTATGCAGT
	TTAAGTACTT	GGCCCTCCTT	TATGATAACATT	TACACATACAGTCA
51 GGCAACATGG	CTTGTTGGATCC	ACCTCAGGGTGG	TCTTCACATTAG	
	CGTTGTACCG	AAACAAAC	CTAGGTGGAGTCCCACC	AGAAAAGTGTAAATC
101 AGGATAACA	ACATATTCCCCAA	ACAATACCCAA	TTAAACTTACCA	CACA
	TCCTATTGTTG	TATAAGGGTTGTT	TATGGGTTAATATTG	AAATGGTGT
151 GCGGGTGC	CACTGTGCAAAG	CTACACAAAC	TTATCAGAGCTGTT	CGCGG
	CGCCCACGGT	GACACGTT	TGATGTGTTGAAATAGT	CTCGACAAGCGCC
201 TCGTTAACAA	ACTGGAGCTGATG	TGAGACATGATA	ACCAAGTGTG	CCAA
	AGCAAATTGTTG	ACACTCGACTAC	CTGTACTATATGGT	CACAACGGTT
251 ACAGAGTTGG	TTGCCTATAAACCA	ACGGTTATT	TTTAGTTAGTTG	AACTCTCA
	TGTCTCAACCA	ACGGATATTG	GGTGC	AAATAAAACTCAACTTGAGAGT
301 AATCATGCAGAG	CTTTCTGTTACAT	ATTAGCGCTGG	ATGTCA	CCAATGCATA
	TTAGTACGTCTCG	AAAGACA	ATGTA	TCGCGACCTACAGTGGTTACGTAT
351 TGTGGTCGG	CTACCGTGCTGG	AAATAGCGC	CATATTCTTC	ATCCTGACA
	ACACCA	CGCGATGGCAC	CTTATCGCGT	ATAAAGAAAGTAGGACTGT
401 ATCAGGAAGAT	GCAGAACGAA	ACTCACTC	ATCTTCACTGATG	TGTTCAAAAT
	TAGTCCTT	CTACGTCTCG	TAGTGAGTAGAAAGT	GACTACAAGTTTA
451 CGATATA	CATTGCCTTGGT	GGTAATT	TATGATAGACTTG	AAACAAC
	GCTATATGTA	AGCGGAAACCAC	CTTAATAACTATCTG	AACTTGAAAC
501 TGGTAATCTGAGAG	AAAATATCGAG	TTGGGAAATGGT	CCACTAGAGGAGG	
	ACCATTAGACT	CTCTTT	TAGCTAAC	CCCTTACCAAGGTGATCTCCTCC
551 CTATCTAGCG	CTTTATTACAGT	ACTGGTGG	CACTCAGCTT	CCAACT
	GATAGAGTC	CGGAAATAATA	ATGTCA	ACCGTGAAGGTTGA
601 CTGGCTCG	TTCTTATAATTG	CATCCAA	ATGATTTCAGAAG	CAGCAAG
	GACCGAGCA	AGGAAATATTAA	ACGTAGGTT	ACTAAAGTCTCGTGTTC
651 ATTCCAATAT	ATTGAGGGAGAA	ATGCGCACG	GAGAATTAGGT	ACAACCGGA
	TAAGGTT	TATAACTCC	CTTACCGTGCT	TTAACCATGTTGGCCT
701 GATCTGCACC	AGATCCTAGCG	TAATTACACTG	GAGAATAGTTGGGGAGA	
	CTAGACGTG	GGTCTAGGAT	CGCATTATGTGAA	ACTCTTATCAACCCCTCT
751 CTTTCCACTG	CAATTCAAGAG	CTAACCAAGGAGC	CTTGCTAGTCCAA	AT
	GAAAGGTGAC	GTAAAGTTCTCAG	ATTGGTCC	CTCGGAAACGATCAGGTTA
801 TCAACTG	CAAAGACG	TAATGGTCCAA	ATTTCAGTGT	TACGATGTGAGTA
	AGTTGACG	TTCTGCATTACCA	AGGTTAAGTC	ACACATGCTACACTCAT
851 TATTAATCC	CTATCATAGCTC	CATGGTGT	TAGATGCGCAC	CTCCACCA
	ATAATTAGGG	ATAGTATCGAGAG	TACCAACATATCTAC	CGCGTGGAGGTGGT
901 TCGTCACAG	TTGCTTGAGAGA	ACGTTCC	TGTGTTCC	CTACTAATGC
	AGCAGTGT	AAACGAAAC	CTCTT	CGCAAGGACAGCAAGGGATGATTACG

51/254

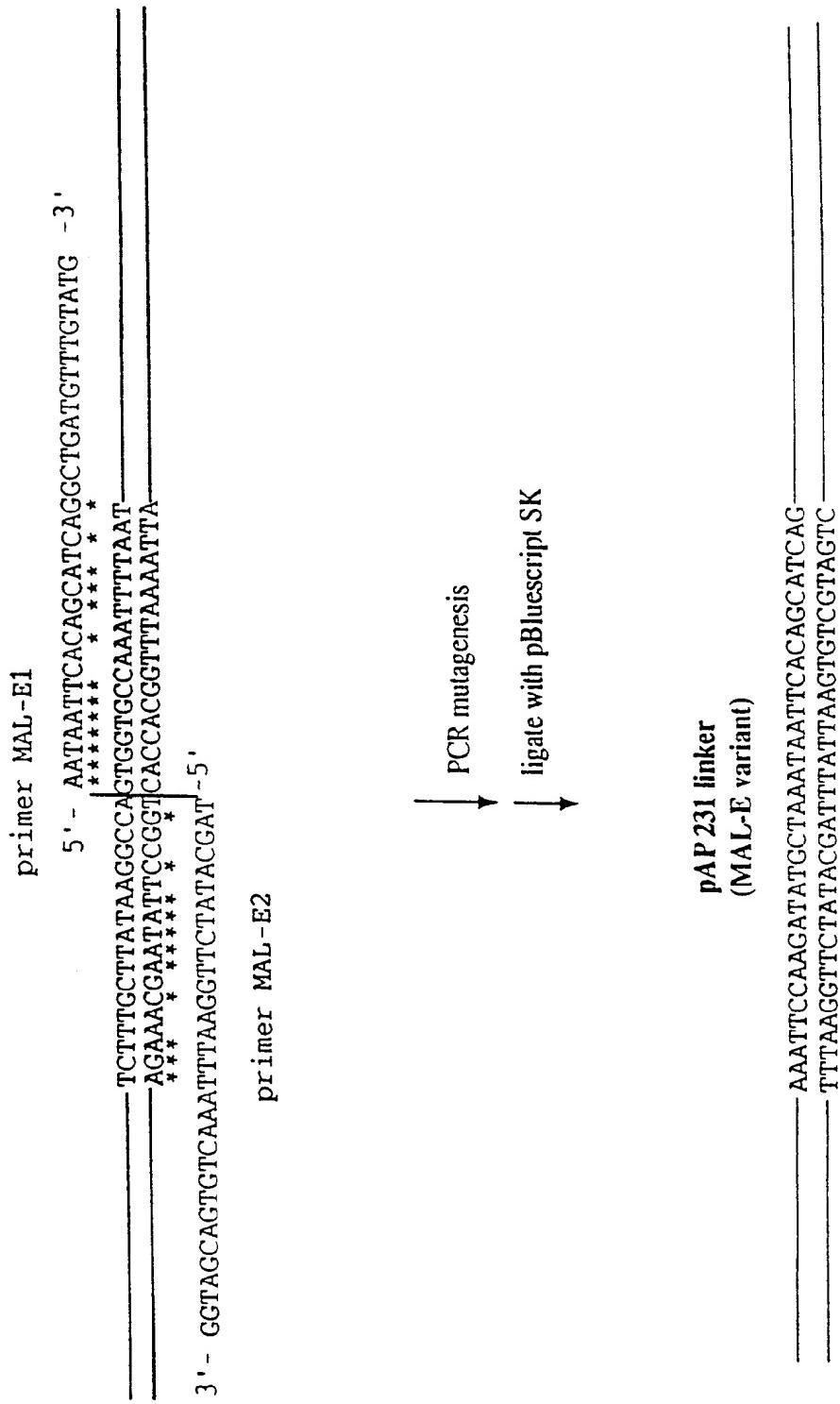
**FIGURE 10D (CONT'D)**

951 TGATGTTGTATGGATCCTGAGCCCATAGTGCCTACGTAGGTCGAAATG  
 ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
 CAGATACACAACATACAATCCCTACCTCTAAGGTGTTGCGCTTGCCTTAC  
  
 1051 CAGTTGTGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTAGATTATGTCAGTTAGTCGAGACCTGAAA  
  
 1101 GAAAAGAGACAATACTATTGATCTAATGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTACAAATTGATGAATGC  
  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA  
 CCATGTCAGGCCCTCAGATACACTAGATACTAACGTTATGACGACGT  
  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCACATCATAAATCC  
 TGACTACGGTGGGGGACCGTTATACCTTACCTGGTAGTATTAGG  
  
 1251 CAGATCTAGTCTAGTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
 GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTCACCATGGTGTG  
  
 1301 TTACAGTGCAAACCAACATTATGCCGTTACTCAAGGTTGGCTTCACT  
 AATGTCACGTTGTTGTAACACGGCAATCAGTCCAACCGAAGGATGA  
  
 1351 AATAATACACAACCTTTGTACAACCATTGTTGGCTATATGGTCTGTG  
 TTATTATGTGTTGAAAACAATGTTGTAACAACCCGATATACCAAGACAC  
  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGGACTGTAGCAGTAAA  
 GAACGTTGCTTATCACCTGTTACACTATCTCCTGACATCGTCACTT  
  
 1451 AGGCTGAACAAACAGTGGGCTTTATGCAGATGGTCAATACTGTCCTCAG  
 TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGAATTCTAATATACGGGAAACAGT  
 GTTTGGCTCTATTAAACGGAATGTTACACTAAGATTATATGCCCTTGTCA  
  
 1551 TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
 ACAATTCTAGGAGAGAACACCCGGGACGTAGGAGACCGGTTGCTACCTACA  
  
 1601 TCAAGAATGATGGAACCATTAAATTGTATAGTGGATTGGTGTAGAT  
 AGTTCTACTACCTGGTAAATTAAACATATCACCTAACCAATCTA  
  
 1651 GTGAGGCGATGGATCCGAGCCTTAAACAAATCATTCTTACCCCTCTCCA  
 CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
  
 1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTGATAGACAGATTACT  
 ACCACTGGGTTGTTACCAATGGTAATAAAACTATCTGTCTAATGA  
  
 1751 CTCTTGAGTGTGTGTCCTGCCATGAAAAATAGATGGCTTAAATAAAA  
 GAGAACGTACACACACAGGACGGTACTTTATCTACCGAATTATTTT  
  
 1801 GGACATTGTAATTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC  
 CCTGTAACATTAAACATTGACTTCCCTGTCGTTCAATATAGCTTAAGG  
  
 1851 TGCAG  
       ACGTC

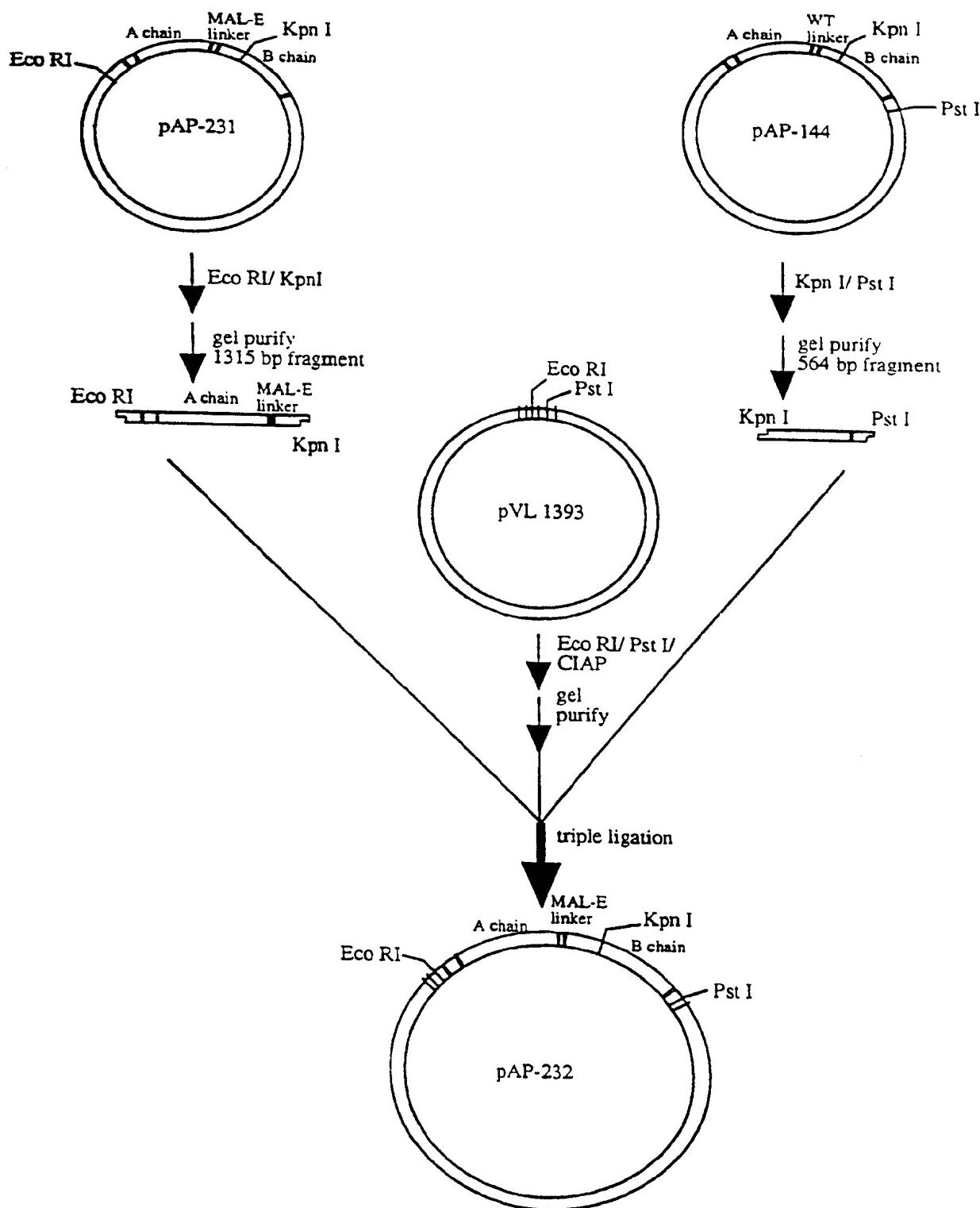
52/254

FIGURE 11A

53 / 254

**FIGURE 11B****WT preprocin linker**

54/254

FIGURE 11C

55/254

**FIGURE 11D**

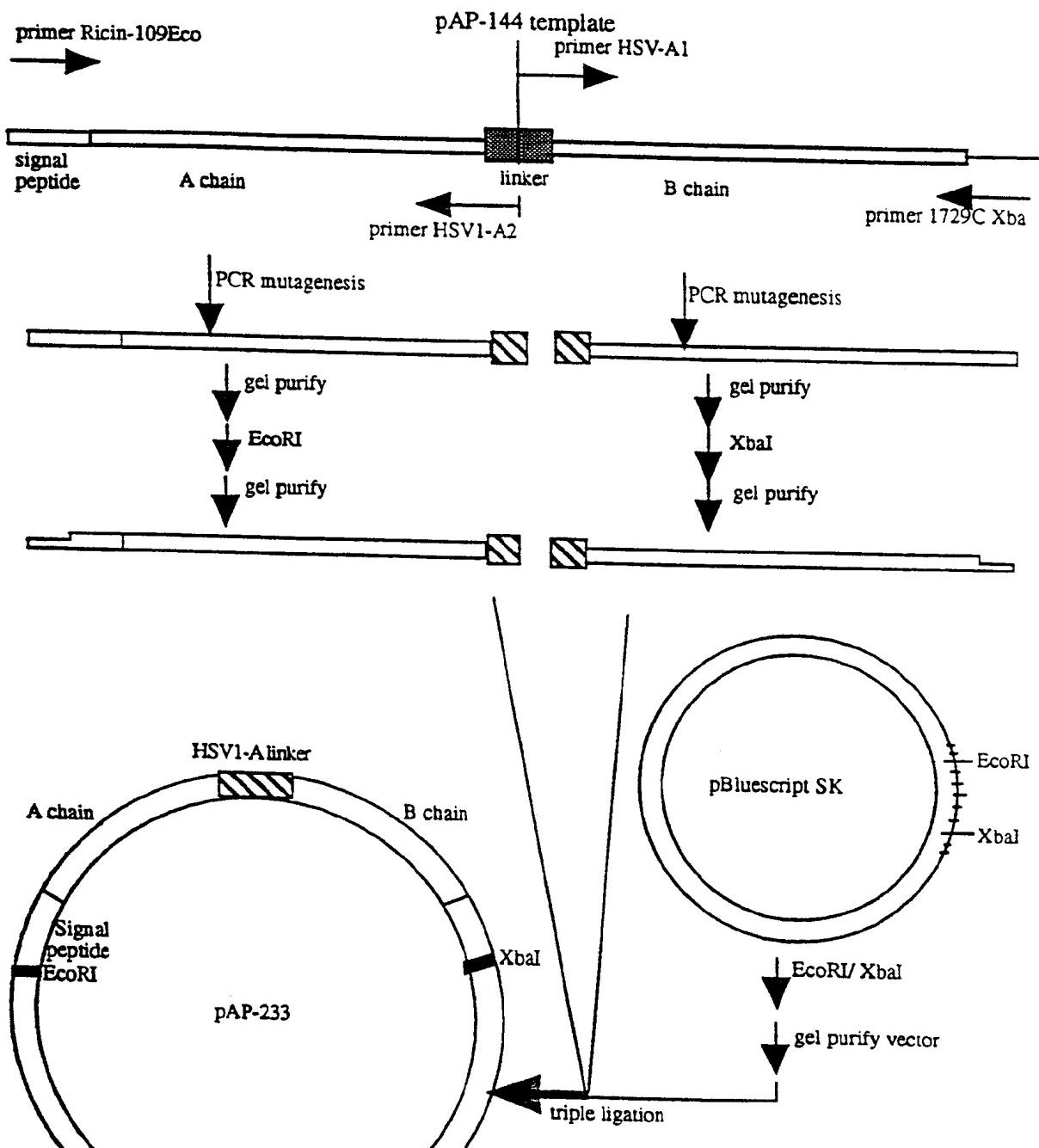
10	20	30	40	50
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT				
CTTAAGTACTTGGCCCTCCTTATGATAACATTATAACCTACATAACGTCA				
51 GGCACACATGGCTTGTGATCCACCTCAGGGTGGCTTTCACATTAG				
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAA				
101 AGGATAACACATATTCCCCAACAAATACCCATTATAACTTACCA				
TCCTATTGTTGATAAGGGTTGTTATGGGTTAATATTGAAATGGTGT				
151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTTATCAGAGCTGTTCGCG				
CGCCACGGTGACACGTTCGATGTGTTGAAATAGTCTCGACAAGGCC				
201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCACTGTTGCCAA				
AGCAAATTGTTGACCTCGACTACACTCTGACTATATGGTCACAACGGTT				
251 ACAGAGTTGGTTGCCATAAACCAACGGTTATTTAGTTGAACCTCTCA				
TGTCTAACCAACCGGATATTGGTGCCTAAATCAACTTGAGAGT				
301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA				
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT				
351 TGTGGTCGGCTACCGTGTGGAAAATAGCGCATATTCTTCATCCTGACA				
ACACCAGCCGATGGCACGCCCTTATCGCGTATAAAGAAAGTAGGACTGT				
401 ATCAGGAAGATGCAGAACAACTCACTCATTTCACTGATGTTCAAAAT				
TAGTCCTCTACGTCTCGTTAGTGAAGTAGAAAGTAGACTACAAGTTTA				
451 CGATATACATCGCCTTGGTGGTAATTATGATAGACTGAAACAACCTG				
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACCTGTTGAACG				
501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG				
ACCATTAGACTCTCTTATAGCTAACCCCTTACCAAGGTGATCTCCTCC				
551 CTATCTCAGCGTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAAC				
GATAGAGTCGCAAATAATAATGTCATGACCAACCGTGAGTCGAAGGTTGA				
601 CTGGCTCGTCTTATAATTGATCCAAATGATTCAGAAGCAGCAAG				
GACCGAGCAAGGAAATTAAACCGTAGGTTACTAAAGTCTCGTCGTTC				
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA				
TAAGGTTATATAACTCCCTCTTACCGTGTCTTAATCCATGTTGGCCT				
701 GATCTGCACCAAGATCCTAGCGTAATTACACTTGAGAAATAGTTGGGGAGA				
CTAGACGTGGTCTAGGATCGCATTAATGTAACACTCTTATCAACCCCTCT				
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT				
GAAAGGTGACGTTAAGTCTCAGATTGGTCCCGGAAACGATCAGGTTA				
801 TCAACTGCAAAGACGAATGGTCCAAATTCACTGATGTGAGTA				
AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT				
851 TATTAATCCCTATCATAGCTCTCATGGTGTAGATGCGCACCTCCACCA				
ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACCGTGGAGGTGGT				
901 TCGTCACAGTTAAATTCAAGATATGCTAAATAATTCAACAGCATCAGGC				
AGCAGTGTCAAATTAAAGGTTCTACGATTATTAAGTGTGCGTAGTCGG				

56/254

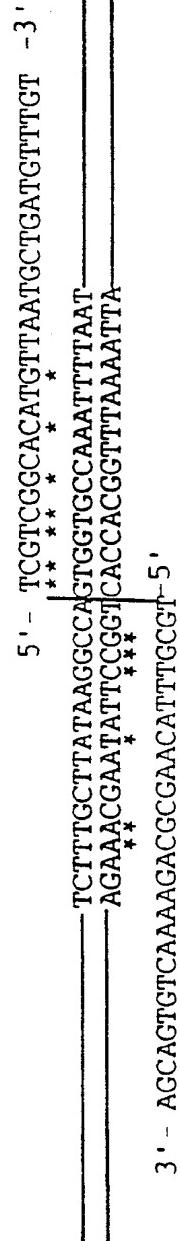
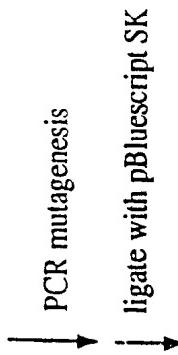
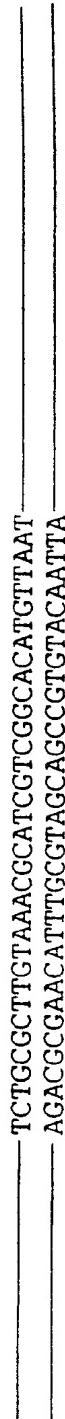
**FIGURE 11D (CONT'D)**

951 TGATGTTGTATGGATCCTGAGCCCATACTGCGTATCGTAGGTCGAAATG  
     ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC  
  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
     CAGATACACAACATACTACCCCTACCTTCTAAGGTGTTGCCTTGCGTTAT  
  
 1051 CAGTTGTGCCATGCAAGTCTAATAACAGATGCAAATCAGCTCTGGACTTT  
     GTCAACACCGGTACGTTACGATTATGTCTACGTTAGTCGAGACCTGAAA  
  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
     CTTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA  
     CCATGTCAGGCCCTCAGATAACACTAGATACTAACGTTATGACGACGT  
  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCACATCAAATCC  
     TGACTACGGTGGCGACCGTTATACCCATTACCTTGGTAGTATTAGG  
  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
     GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTCACCATGGTGTG  
  
 1301 TTACAGTGCAAACCAACATTATGCCGTTAGTCAGGTTGGCTTCTACT  
     AATGTCACGTTGGTTGTAACGGCAATCAGTCCAACCGAAGGATGA  
  
 1351 AATAATAACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
     TTATTATGTGTTGGAAAACAATGTTGGTAAACAACCGATATAACCAGACAC  
  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGA  
     AAACGTTGTTATCACCTGTTACACTATCTCTGACATCGTCACTTT  
  
 1451 AGGCTGAACAACAGTGGCTTTATGCAGATGGTCAATAACGTCCCTCAG  
     TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGAATTCTAATATAACGGAAACAGT  
     GTTTGCGCTATTAACGGAATGTTACTAAGATTATATGCCCTTGTCA  
  
 1551 TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
     ACAATTCTAGGAGAGAACACCCGGACGTAGGAGACCGGTTGCTACCTACA  
  
 1601 TCAAGAATGATGGAACCATTAAATTGTATAGTGGATTGGTGTAGAT  
     AGTTCTACTACCTGGTAAATTAAACATATCACCTAACCAATCTA  
  
 1651 GTGAGGGCGATCGGATCCGAGCCTAAACAAATCATTCTTACCCCTCTCCA  
     CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
  
 1701 TGGTACCCAAACCAAATATGGTTACCAATTATTTGATAGACAGATTACT  
     ACCACTGGGTTGGTTATACCAATGGAATAAAACTATCTGTCTAATGA  
  
 1751 CTCTTGCACTGTGTGTCCTGCCATGAAAATAGATGGCTAAATAAAAAA  
     GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATTTT  
  
 1801 GGACATTGTAATTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC  
     CCTGTAACATTAAAACATTGACTTTCTGTCGTTCAATATAGCTTAAGG  
  
 1851 TGCAG  
     ACGTC

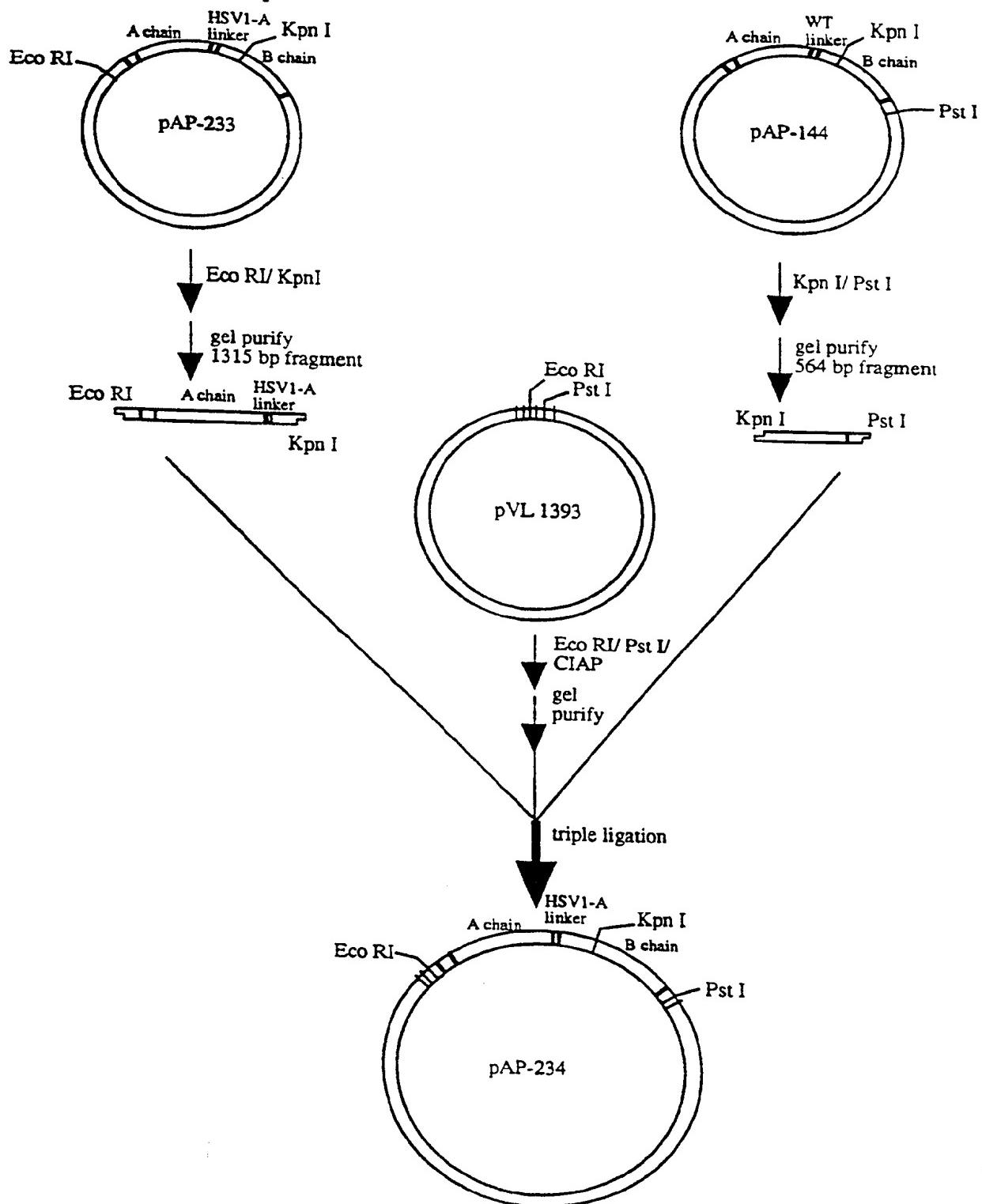
57/254

**FIGURE 12A**

58/254

**FIGURE 12B****WT preprorcin linker****primer HSV1-A****primer HSV1-A****pAP233 linker  
(HSV1-A variant)**

59/254

**FIGURE 12C**

60/254

**FIGURE 12D**

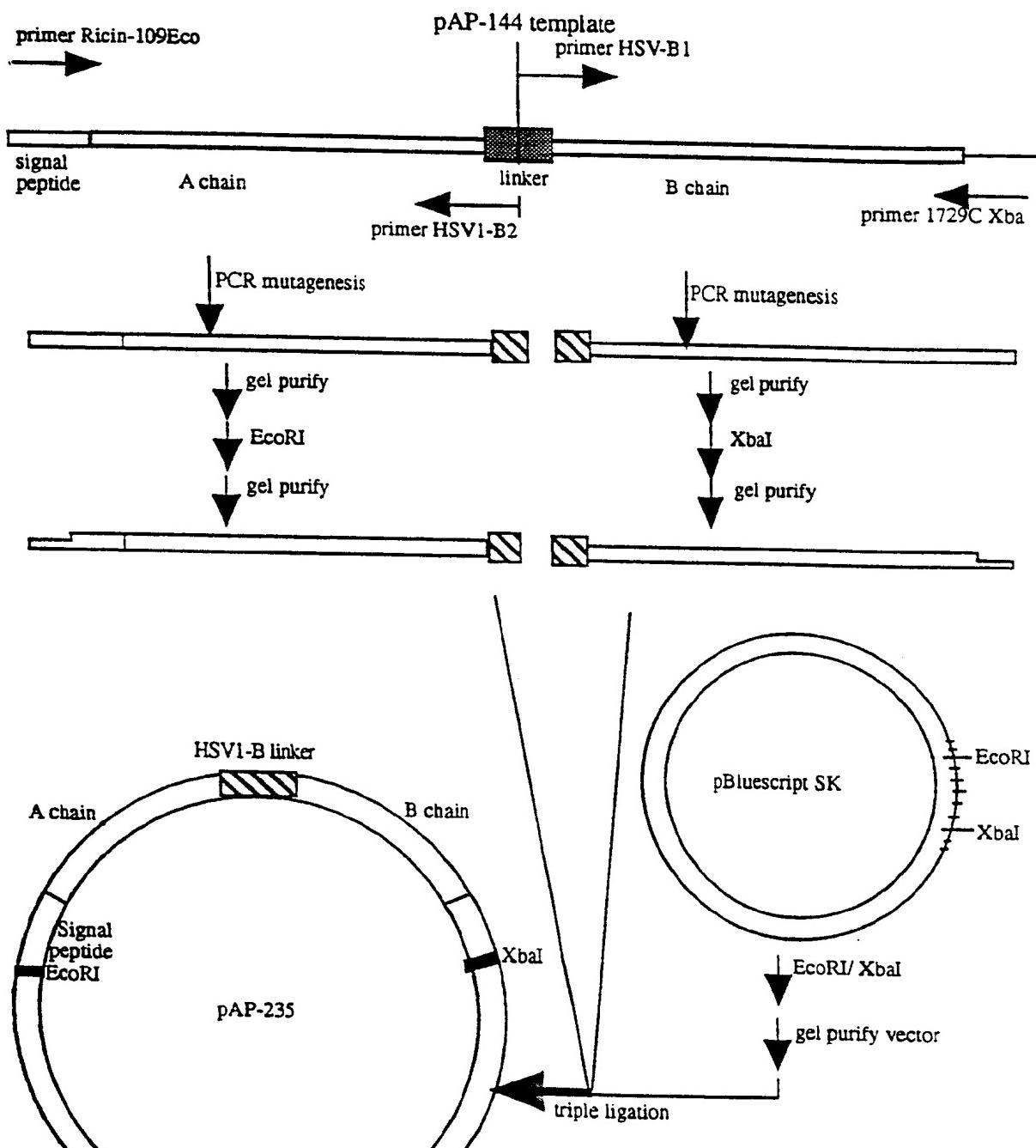
10	20	30	40	50
1 GAATT CATGAAACCGGGAGGAAAATACTATTGTAAATATGGATGTATGCAGT				
CTTAAGTACTTTGGCCCTCTTATGATAACATTATACTACATACGTCA				
51 GGCAACATGGCTTGTTGGATCCACCTCAGGGTGGTCTTCACATTAG				
CCGTTGTACCGAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAA				
101 AGGATAACAAACATATTCCCCAAACAATACCCAATTATAAACCTTACCA				
TCCTATTGTTGATAAGGGGTTGTTATGGTTAATATTGAAATGGTGT				
151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTTATCAGAGCTGTTCGCGG				
CGCCCAACGGTACACGTTCGATGTGTTGAAATAGTCTCGACAAGGCC				
201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCAAGTGTGCCAA				
AGCAAATTGTTGACCTCGACTACACTCTGACTATATGGTCACAACGGTT				
251 ACAGAGTTGGTTGCCTATAAACCAACGGTTTTAGTTAGTTGAAC				
TGTCTCAACCAAACGGATATTGGTTGCCAAATAAAACTCAACTTGAGAGT				
301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA				
TTAGTAGCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT				
351 TGTGGTCGGCTACCGTGTGGAAATAGCGCATATTCTTCATCCTGACA				
ACACCAGCGATGGCACGACCTTATCGCGTATAAAAGAAAGTAGGACTGT				
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTCACTGATGTTCAA				
TAGTCCTTCTACGTCTCGTTAGTGAGTAGAAAGTGA				
451 CGATATACATTGCCCTTGGTGTAAATTATGATAGACTTGAACAACTTGC				
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACCTGTTGAACG				
501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG				
ACCATTAGACTCTTTATAGCTAACCCCTTACAGGTGATCTCCTCC				
551 CTATCTCAGCGCTTTATTACAGTACTGGTGGCACTCAGCTTCAA				
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA				
601 CTGGCTCGTTCTTATAATTGCA				
CATCCAAATGATTCAGAAGCAGCAAG				
GACCGAGCAAGGAAATATTAACGTAGGTTACTAAAGTCTCGTGTTC				
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAAATTAGGTACAACCGGA				
TAAGGTTATATAACTCCCTTTACCGTGTCTTAATCCATGTTGGCCT				
701 GATCTGCACCAAGATCCTAGCGTAATTACACTTGAGAAATAGTTGGGGAGA				
CTAGACGTGGTCTAGGATCGCATTAAATGTGA				
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCAA				
GAAAGGTGACGTTAAGTCTCAGATTGGTCTCGGAAACGATCAGGTTA				
801 TCAACTGCAAAGACGTAATGGTCAAATTCAAGGTAAAGTCAACACATGCTACACTCAT				
851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA				
ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACCGTGGAGGTG				
901 TCGTCACAGTTCTCGCCTGTAACCGCATCGTGGCACATGTTAATGC				
AGCAGTGTCAAAAGACGCGAACATTGCGTAGCAGCCGTGTACAATTACG				

61/254

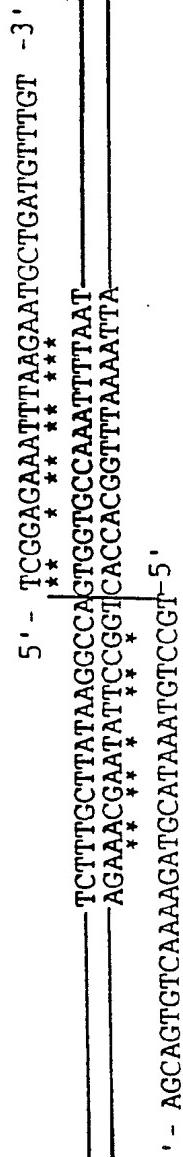
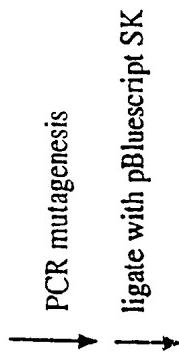
FIGURE 12D (CONT'D)

951 TGATGTTGTATGGATCCTGAGCCCATACTGCCTACGTAGGTCGAAATG  
 ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
 CAGATACACAACATACTACATCCCTACCTCTAAGGTGTTGCCCTTGCCTTAT  
 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTCAAGATTATGTCAGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCGGGAGTCTATGTGATGATCTATGATTGCAAATACTGCTGCA  
 CCATGTCAGGCCCTCAGATACTACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC  
 TGACTACGGTGGCGACCGTTTACCTTACCTTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
 GTCTAGATCAGATCAAATCGTCGCTGTAGTCCTGTACCATGGTGTG  
 1301 TTACAGTCAAACCAACATTATGCCGTTAGTCAGGTTGGCTTCAACT  
 AATGTCACGTTGGTTGTAATAACGGCAATCAGTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
 TTATTATGTTGGAAAACAATGTTGTAACAACCCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGGACTGTAGCAGTGAA  
 GAACGTTCGTTTACCTGTTACACCTATCTCCTGACATCGTCACCTT  
 1451 AGGCTGAACAAACAGTGGGCTTTATGCAGATGTTCAATACTGCCTCAG  
 TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGATTCTAATATAACGGAAACAGT  
 GTTTGGCTCTATTAAACGGAATGTTCACTAAGATTATATGCCCTTGCA  
 1551 TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
 ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA  
 1601 TCAAGAATGATGGAACCATTAAATTGTTAGTGGATTGGTGTAGAT  
 AGTTCTTACTACCTTGGTAAATTAACATATCACCTAACCAACAACTCTA  
 1651 GTGAGGCGATCGGATCCGAGCCTAAACAAATCATTCTTACCCCTCTCCA  
 CACTCCCGTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
 1701 TGGTGACCCAAACCAAATATGGTTACCAATTGTTAGACAGACGATTACT  
 ACCACTGGGTTGGTTACCAATGTAATAAAACTATCTGTCTAATGA  
 1751 CTCTTGCAGTGTGTGTCTGCCATGAAAATAGATGGCTAAATAAAAAA  
 GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATT  
 1801 GGACATTGTAATTGTAACTGAAAGGACAGCAAGTTATCGAATTCC  
 CCTGTAACATTAAACATTGACTTCTGTGTTCAATATAGCTTAAGG  
 1851 TGCAG  
       ACGTC

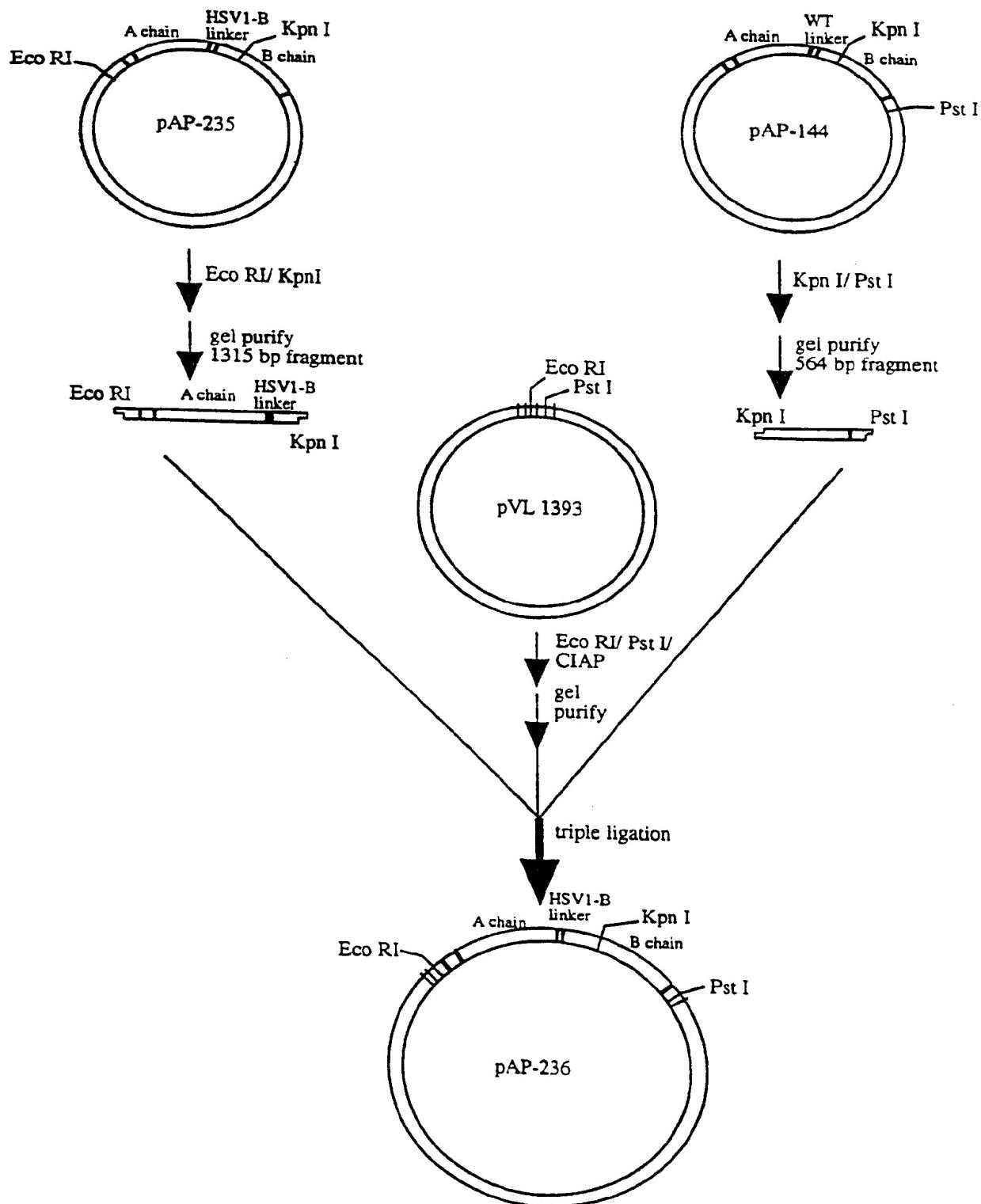
62/254

**FIGURE 13A**

63/254

**FIGURE 13B****WT preprorcin linker****primer HSV1-B****primer HSV1-B****pAP235 linker  
(HSV1-B variant)**

64/254

FIGURE 13C

65 / 254

**FIGURE 13D**

10            20            30            40            50

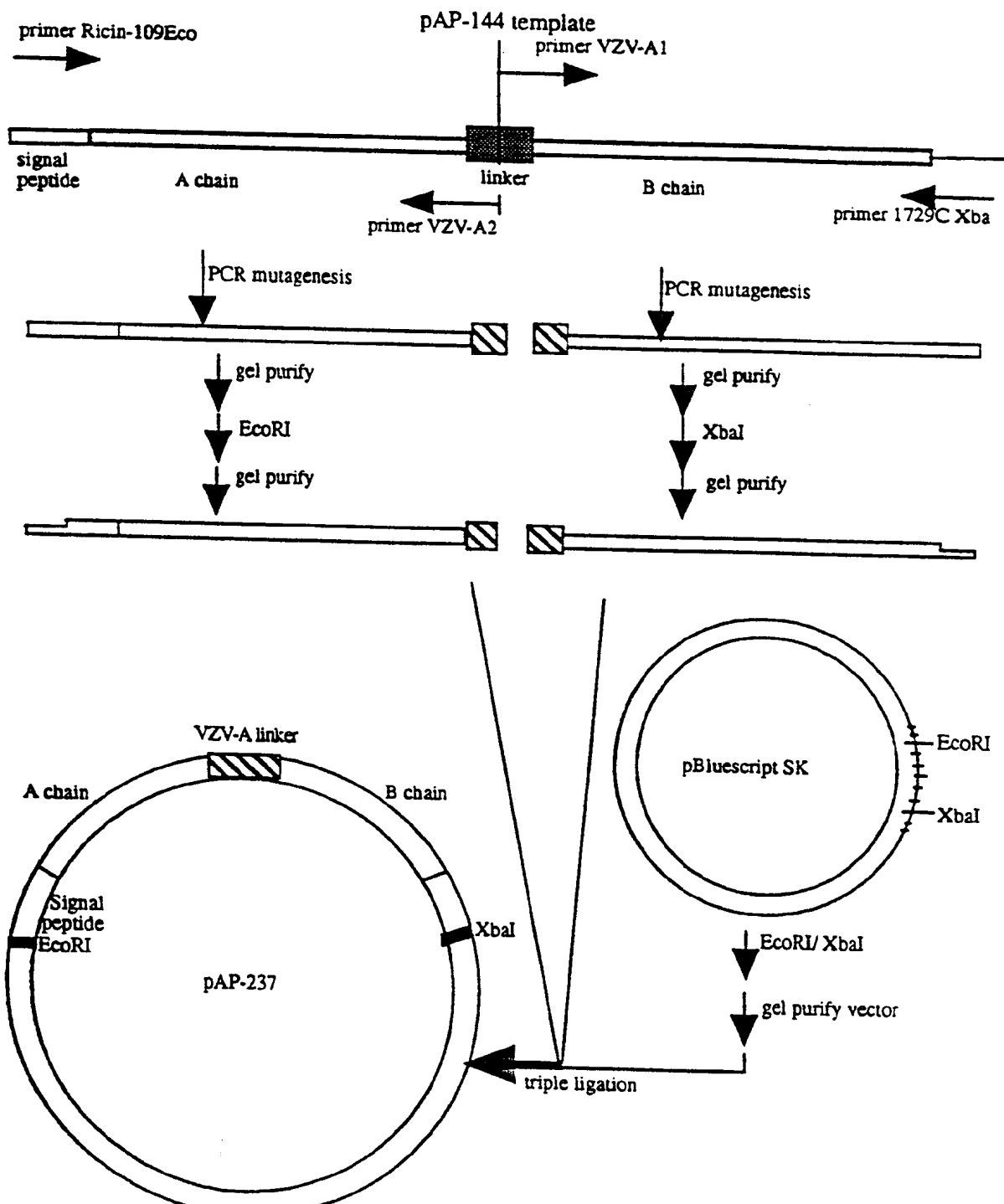
1 GAATTCATGAAACGGGGAGGAAATACTATTGTAATATGGATGTATGCAGT  
 CTTAAGTACTTGGCCCTCCTTATGATAACATTATACCTACATACGTCA  
 51 GGCAACATGGCTTGTGATCCACCTCAGGGTGGCTTTCACATTAG  
 CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC  
 101 AGGATAACAACATACTCCCCAAACAAATAACCCAAATTATAAACTTTACCA  
 TCCTATTGTTGTATAAGGGGTTGTTATGGTTAATATTGAAATGGTGT  
 151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTTATCAGAGCTGTCGCC  
 CGCCCACGGTGACACGTTGATGTGTTGAAATAGTCTCGACAAGGCC  
 201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATAACCAGTGGCCAA  
 AGCAAATTGTTGACCTCGACTACACTCTGACTATATGGTCACAACGGTT  
 251 ACAGAGTTGGTTGCCCTATAAAACCAACGGTTATTAGTTAGTTGAACCTCA  
 TGTCTCAACCAAACGGATATTGGTTGCAAATAAAATCAACTTGAGAGT  
 301 AATCATGCGAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA  
 TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT  
 351 TGTGGTCGGCTACCGTGTGAAATAGCCATATTCTTACCTGACA  
 ACACCAAGCGATGGCACGACCTTATCGGTATAAAGAAAGTAGGACTGT  
 401 ATCAGGAAGATGCGAGCAATCACTCATCTTTCACTGATGTTCAAAAT  
 TAGTCCTCTACGTTCTCGTTAGTAGAAAGTAGTACAAAGTTTA  
 451 CGATATACATTGCTTTGGTGGTAATTATGATAGACTTGAACAAACTTGC  
 GCTATATGTAAGCGAAACCAACCTTAATACTATCTGAACCTGTTGAACG  
 501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG  
 ACCATTAGACTCTCTTATAGCTAACCTTACCGGTGATCTCCTCC  
 551 CTATCTAGCGCTTATTACAGTACTGGTGGCACTCAGCTTCCA  
 GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA  
 601 CTGGCTCGTCTTATAATTGCAATCCAAATGATTCAGAACCGAGCAAG  
 GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTCGTT  
 651 ATTCCAATATATTGAGGGAGAAAATGCGCACGAGAAATTAGGTACAACCGGA  
 TAAGGTTATATAACTCCCTTTACCGTGCTTTAATCCATGTTGGCCT  
 701 GATCTGCAACAGATCTAGCGTAATTACACTTGAGAAATAGTTGGGGAGA  
 CTAGACGTGGCTAGGATCGCATTATGTAACCTTATCAACCCCCCTCT  
 751 CTTTCCACTGCAATCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT  
 GAAAGGTGACGTTAAGTCTAGATTGGTCTCGGAAACGATCAGGTTA  
 801 TCAACTGCAAAGACGTAATGGTCCAAATTCACTGAGTGTGAGTA  
 AGTTGACGTTCTGCAATTACCAAGGTTAACGTACACATGCTACACTCAT  
 851 TATTAATCCCTATCATAGCTCTCATGGGTATAGATGCGCACCTCCACCA  
 ATAATTAGGGATAGTATCGAGAGTACCCACATATCTACCGTGAGGTGGT  
 901 TCGTCACAGTTTCTACGTATTTACAGGCATCGGAGAAAATTAAAGAATGC  
 AGCAGTGTCAAAAGATGCATAATGTCGTTAGCCTTTAAATTCTTACG

66/254

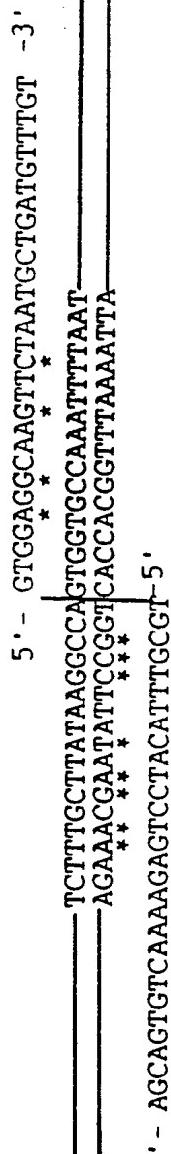
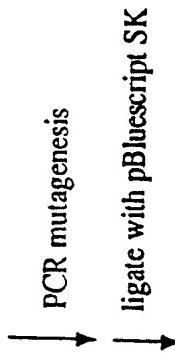
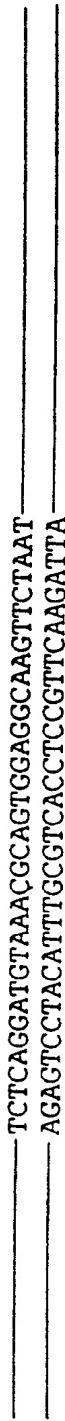
**FIGURE 13D (CONT'D)**

951 TGATGTTGTATGGATCCTGAGCCCATA GTGCGTATCGTAGGTCGAAATG  
 ACTACAAACATA CCTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
 CAGATACACAACATA CCTACCTTAAGGTGTTGCCTTGCGTTAT  
 1051 CAGTTGTGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTCA GATTATGCTACGTTAGTCAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA  
 CCATGTCAGGCCCTCAGATACTACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC  
 TGACTACGGTGGCGACCGTTATACCTTACCTTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
 GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTCACCATGGTGTG  
 1301 TTACAGTGCACCAACACATTATGCCGTTAGTCAAGGTTGGCTTCTACT  
 AATGTCACGTTGGTTGTAACGGCAATCAGTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
 TTATTATGTTGAAAACATGTTGGTAACAACCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
 GAACGTTCGTTATCACCTGTTACACCTATCTCCTGACATCGTCACCTT  
 1451 AGGCTGAACAAACAGTGGCTCTTATGCAAGATGGTTCAATACGTCTCAG  
 TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGATTCTAATATACGGGAAACAGT  
 GTTTGGCTCTATTACGGAATGTTCACTAAGATTATATGCCCTTGTC  
 1551 TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
 ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA  
 1601 TCAAGAATGATGGAACCATTAAATTGTATAGTGGATTGGTGTAGAT  
 AGTTCTTACTACCTTGGTAAATTTAAACATATCACCTAACCAATCTA  
 1651 GTGAGGGCGATCGGATCCGAGCCTAAACAAATCATTCTTACCCCTCTCCA  
 CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
 1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTGATAGACAGATTACT  
 ACCACTGGGTTGGTTATACCAATGGAATAAAACTATCTGCTAATGA  
 1751 CTCTTGCAGTGTGTCCTGCCATGAAAATAGATGGCTAAATAAAAAA  
 GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATT  
 1801 GGACATTGTAATTTGTAACTGAAAGGACAGCAAGTTATATGAAATTCC  
 CCTGTAACATTAAAACATTGACTTTCTGTCGTTCAATAAGCTTAAGG  
 1851 TGCAG  
 ACGTC

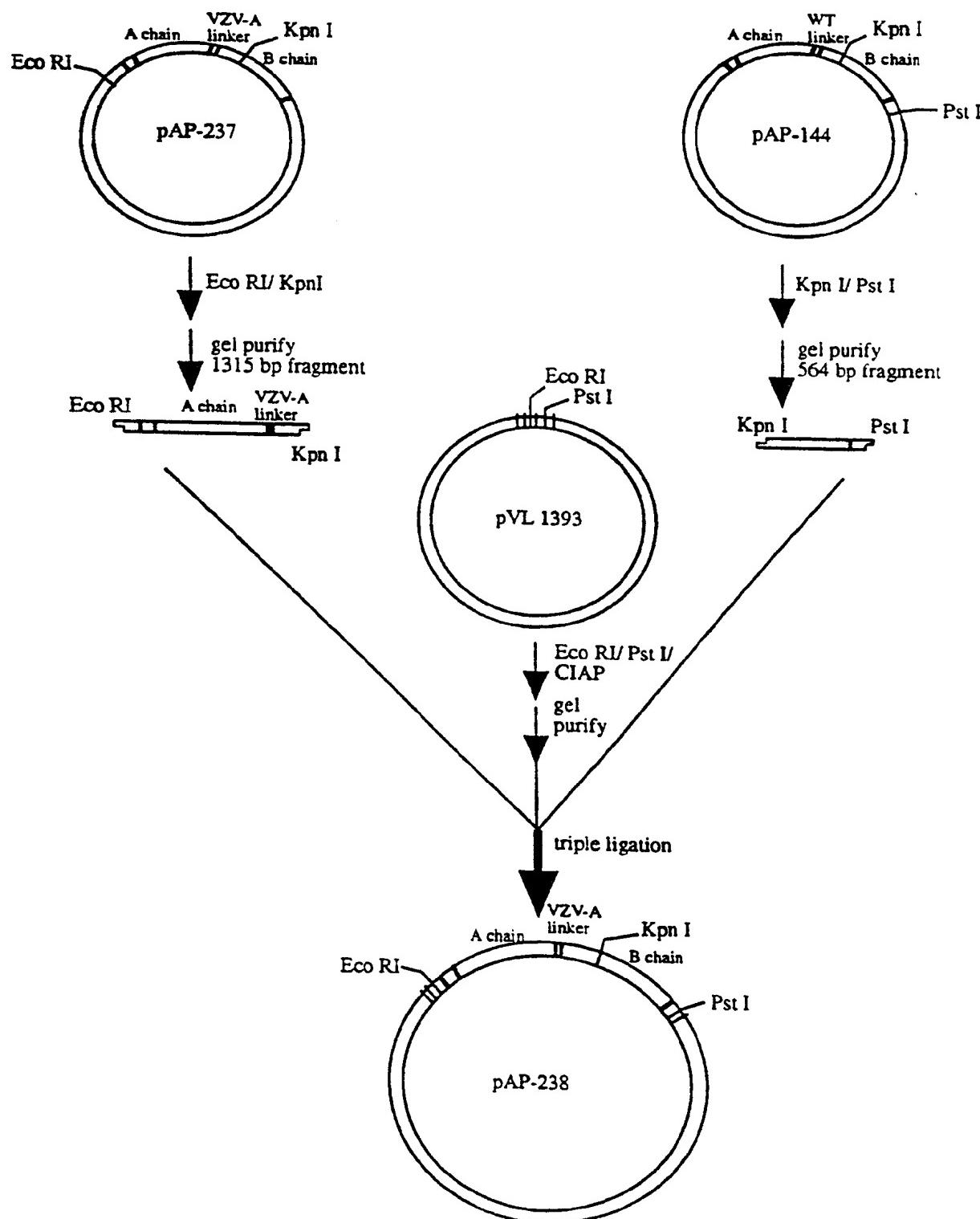
67/254

**FIGURE 14A**

68/254

**FIGURE 14B****WT preprorcin linker****primer VZV-A1****primer VZV-A2****pAP 237 linker  
(VZV-A variant)**

69/254

**FIGURE 14C**

70/254

FIGURE 14D

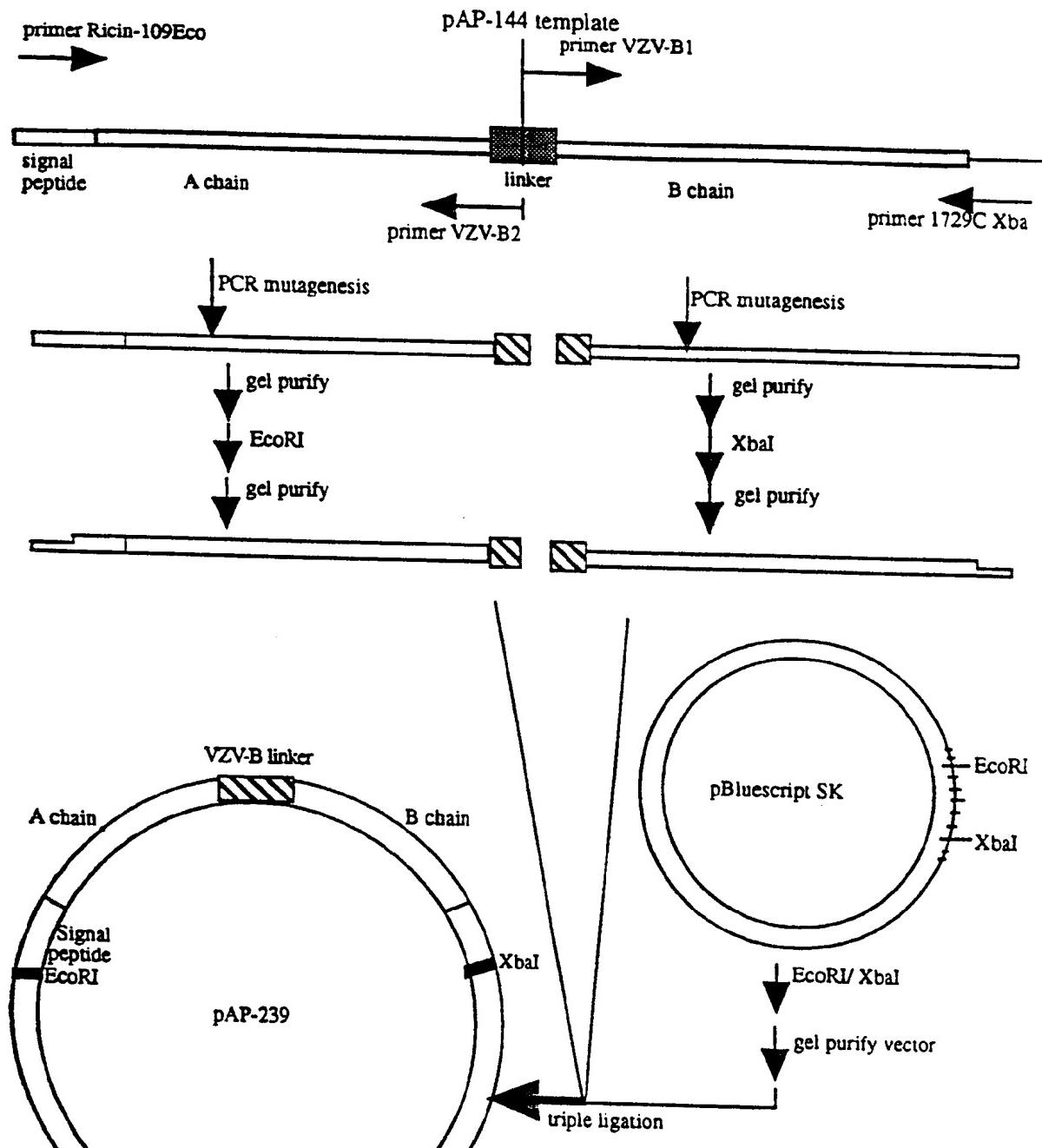
10	20	30	40	50
1 GAATT	CATGAA	ACCGGGAGGGAA	ATACTATTGT	ATATGGATGTATGCAGT
				CTTAAGTACTT
51 GGCAACATGG	CTTGTTGGATCC	ACCTCAGGGTGGT	CTTCACATTAG	
	CCGTTGTACCGAA	ACAAA	CTAGGTGGAGTCCCACCAGAAAGTGTAA	ATC
101 AGGATAACA	ACATATTCCCCAA	ACAATACCA	AACTTAAACTTACCA	
	TCCTATTGTTG	TATAAGGGTTGTT	ATGGGTTAATATTGAA	ATGGTGT
151 GCGGGTGCC	ACTGTGCAA	AGCTACACAA	ACCTTATCAGAGCTGTT	CGCG
	CGCCCACGGTGAC	ACGTTGATGTGTT	GAAATAGTCTCGACAAGCGCC	
201 TCGTTAACAA	ACTGGAGCTGATGT	GAGACATGATA	ACCAACTTACAGTGTG	CCAA
	AGCAAATTGTTG	ACCTCGACTACACTCTG	TACTATATGGTCACAACGGTT	
251 ACAGAGTTGG	TTGCCTATAAACCA	ACGGTT	TTAGTTAGTTGA	ACTCTCA
	TGTCTCAACCA	ACGGATATTGTTG	CCAAATAAA	ACTTGAGAGT
301 AATCATGCAGAG	CTTCTGTTACATTAG	CGCTGGATGT	CACCAATGCATA	
	TTAGTACGTC	CGAAAGACAATGT	ATCGCGACCTACAGTGGTT	ACGT
351 TGTGGTCGG	CTACCGTGTGAA	ATAGCGCATA	TTTCTTCATCCTGACA	
	ACACCAGCGATGG	ACGACCTT	ATCGCGTATAAAGAA	AGTAGGACTGT
401 ATCAGGAAGATG	CAGAAGCAATCA	CTCATCTTCA	CTGATGTT	AAAAT
	TAGTCCTTCTACG	TCTCGTTAGT	GAGTAGAAAAGT	GACTACAAGTTTA
451 CGATATACATTG	CCTTGGTGTA	ATTATGATAGACT	TGAACAAC	TTGC
	GCTATATGTAAGCG	AAACCACCA	TTAATACTATCTGAA	CTTGAAAC
501 TGGTAATCTGAGAG	AAAATATCGAG	TTGGGAAATGGTCC	ACTAGAGGAGG	
	ACCATTAGACTCT	TTTATAGCT	CAACC	TTTACCAAGGTGATCTCCTCC
551 CTATCTCAGCG	TTTATTACAGT	ACTGGTGGCA	CTCAGCTTCCA	ACT
	GATAGAGTCG	CGAAATAATA	ATGT	CATGACCACCGTGAGTCGAAGGTG
601 CTGGCTCG	TCTTTATAATTG	CATCCAAATGAT	TTCAAGAAGCAGCAAG	
	GACCGAGCAAGGAA	ATTATAACGTAGGTT	ACTAAAGTCTCGTCGTT	
651 ATTCCAATATATTGAGGGAG	AAATGCGCACGAGA	ATTAGGTACAAACCGGA		
	TAAGGTTATATA	ACTCCCTTTACCGGTG	CTTAATCCATGTTGGCCT	
701 GATCTGACCAG	ATCCTAGCGTA	ATTACACTTGAGA	AAATAGTTGGGGAGA	
	CTAGACGTGGT	CTAGGATCGC	ATTAATGTGAA	CTTATCAACCCCTCT
751 CTTTCCACTGCA	ATTCAAGAGTCTA	ACCAAGGAGC	TTTGCTAGTCCAAT	
	GAAAGGTGACGTTA	AGTTCTCAGAT	GGTCTCGGAAACGATCAGGTTA	
801 TCAACTGCA	AAAGACGTA	ATGGTCAA	ATTCAAGAGTCTA	
	AGTTGAC	TTCTGCATTAC	ACAGGTTAAGTCACACATGCTACACTCAT	
851 TATAAATCC	TATCATAGCT	CTCATGGT	TAGATGCGCACCTCCACCA	
	ATAATTAGGG	ATAGTATCGAGAGT	ACACATATCTACGCGTGGAGGTGGT	
901 TCGTCACAG	TTTCTCAGGATG	TAACGCA	GAGTCTAATGC	
	AGCAGTGTCA	AAAGAGTC	CTACATTGCGT	ACCTCCGTCAAGATTACG

71/254

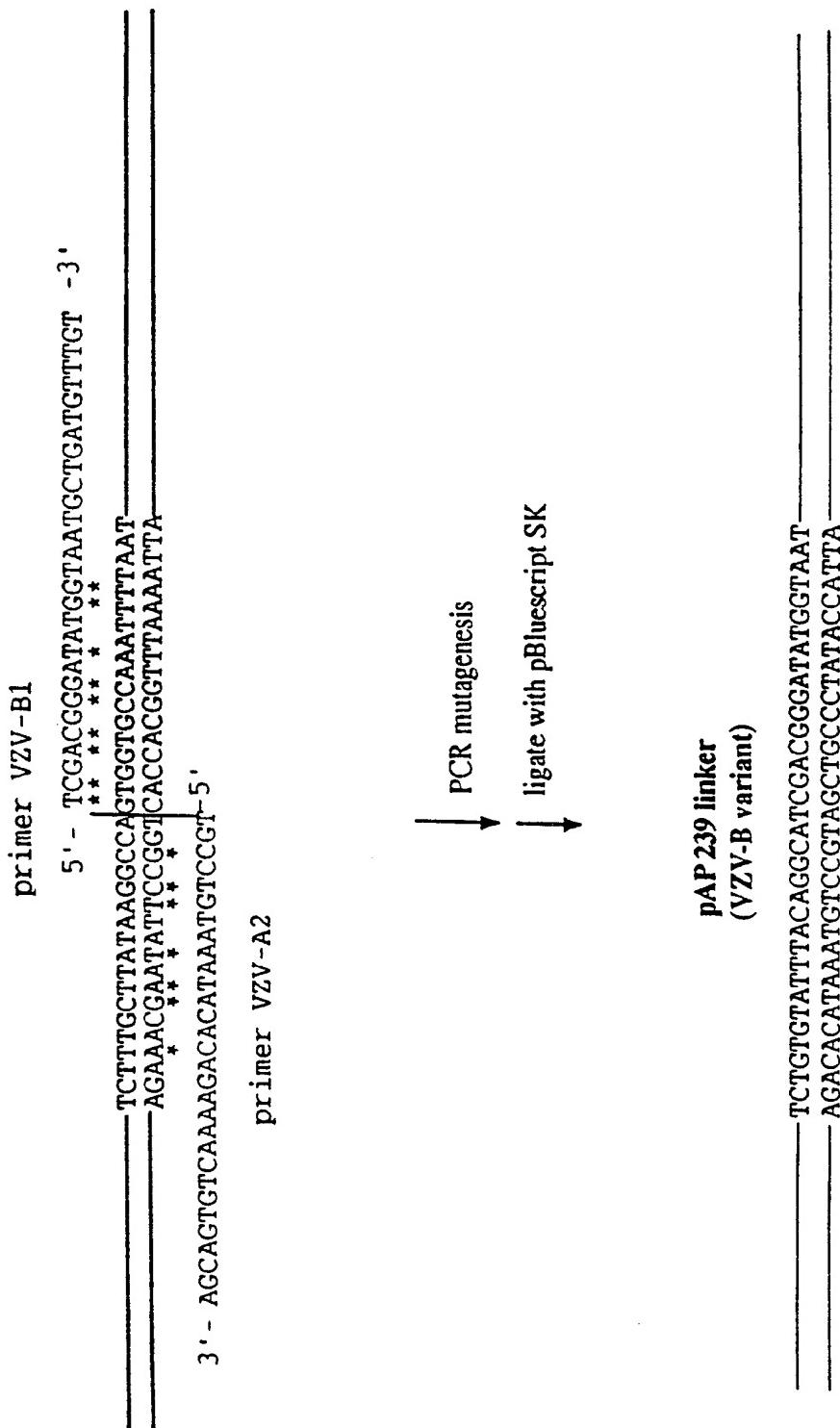
**FIGURE 14D (CONT'D)**

951 TGATGTTGTATGGATCCTGAGCCCATAGTGCCTATCGTAGGTCGAAATG  
 ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGAAACGCAATA  
 CAGATACACAACATACTACCTTACCTCTAAGGTGTTGCCTTGCCTTAT  
  
 1051 CAGTTGTGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTCAGATTATGTCAGCTTACGTTAGTCGAGACCTGAAA  
  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTGTTAACCTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAAATACTGCTGCA  
 CCATGTCAGGCCCTCAGATAACACTAGATAACTAACGTTATGACGACGTT  
  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC  
 TGACTACGGTGGCGACCGTTATACCTTACCTGGTAGTATTAGG  
  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC  
 GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTCACCATGGTGTG  
  
 1301 TTACAGTGCAAACCAACATTATGCCGTTAGTCAAGGTTGGCTTCTACT  
 AATGTCACGTTGTTGTAACACGGCAATCAGTCCAACCGAAGGATGA  
  
 1351 AATAATACACAACCTTTGTTACAACCAATTGTTGGCTATATGGTCTGTG  
 TTATTATGTTGGAAAACAATGTTGTAACAACCCGATATACCAAGACAC  
  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
 GAACGTTCGTTATCACCTGTTACACCTATCTCCTGACATCGTCACTT  
  
 1451 AGGCTGAACAAACAGTGGCTCTTATGCAGATGGTCAATACGTCTCAG  
 TCCGACTTGTGTCACCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGAATTCTAATATACGGGAAACAGT  
 GTTTGGCTCTATTAAACGGAATGTTCACTAAGATTATATGCCCTTGTCA  
  
 1551 TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCAACGATGGATGT  
 ACAATTCTAGGAGAGAACACCGGGACGTTAGGAGACCGGTTGCTACCTACA  
  
 1601 TCAAGAATGATGGAACCATTTAAATTGTTAGTGGATTGGTGTAGAT  
 AGTTCTTACTACCTTGGTAAATTAACATATCACCTAACCAATCTA  
  
 1651 GTGAGGCGATCGGATCCGAGCCTAAACAAATCATTCTTACCCCTCTCCA  
 CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
  
 1701 TGGTGACCCAAACCAAATATGGTTACCAATTATTTGATAGACAGATTACT  
 ACCACTGGGTTGGTTATACCAATGGAATAAAACTATCTGTCTAATGA  
  
 1751 CTCTTGCAGTGTGTGTCCTGCCATGAAAATAGATGGCTAAATAAAAAA  
 GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATTTT  
  
 1801 GGACATTGTAATTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC  
 CCTGTAACATTAAAACATTGACTTTCTGTCGTTCAATATAGCTTAAGG  
  
 1851 TGCAG  
 ACGTC

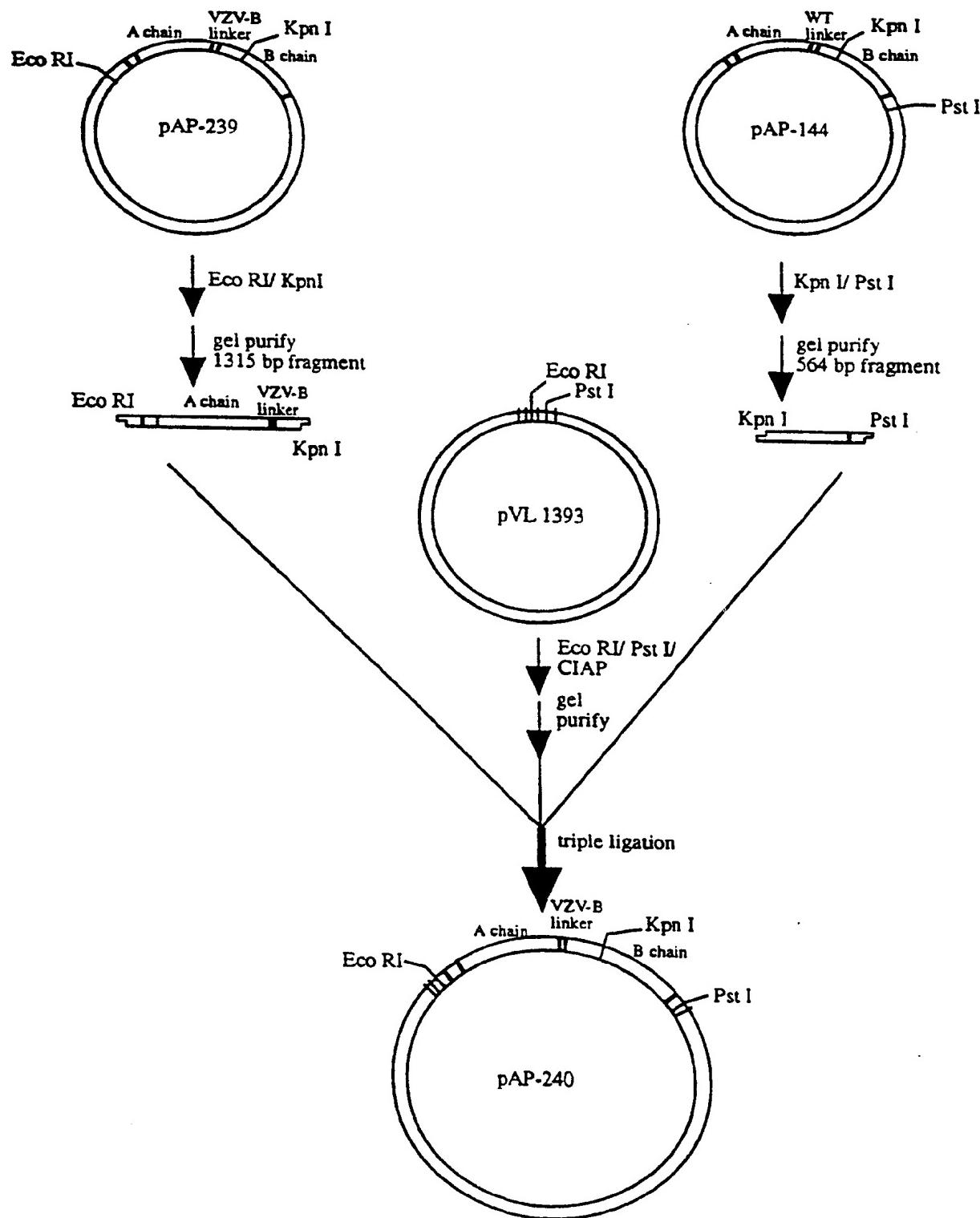
72/254

**FIGURE 15A**

73/254

**FIGURE 15B****WT prorocin linker**

74/254

**FIGURE 15C**

75/254

FIGURE 15D

10	20	30	40	50
1	GAATTCATGAAACCGGGAGAATACTATTGTAATATGGATGTATGCAGT			
	CTTAAGTACTTTGGCCCTCCTTATGATAACATTACACATACGTCA			
51	GGCAACATGGCTTGTTGGATCCACCTCAGGGTGGTCTTCACATTAG			
	CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAA			
101	AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCA			
	ACACCAACCGTTGACACGTTCGATGTGTTGAAATAGTCTCGACAAGGCC			
151	GCGGTGCCACTGTGCAAAGCTACACAAACTTTATCAGAGCTGTTGCCG			
	CGCCCACGGTACACGTTCGATGTGTTGAAATAGTCTCGACAAGGCC			
201	TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCAAGTGTGCAA			
	AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT			
251	ACAGAGTTGGTTGCCTATAACCAACGGTTATTTAGTTGAACCTCTCA			
	TGTCTCAACCAACGGATATTTGGTTGCCAAATAAAACTCAACTTGAGAGT			
301	AATCATGCGAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA			
	TTAGTACGTCTCGAAAGACAAATGTAATCGCACCTACAGTGGTTACGTAT			
351	TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTCATCCTGACA			
	ACACCAAGCCGATGGCACGACCTTATCGCGTATAAAAGAAAGTAGGACTGT			
401	ATCAGGAAGATGCAGAAGCAATCACTCATTTTCACTGATGTTCAAAAT			
	TAGTCCTTCTACGTCTCGTTAGTGAAGTAGAAAAGTGA			
451	CGATATACATTGCCCTTGGTGGTAATTATGATAGACTTGAACAACTTGC			
	GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACCTGTTGAACG			
501	TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG			
	ACCATTAGACTCTTTATAGCTAACCTTACCAAGGTGATCTCCTCC			
551	CTATCTCAGCGTTTATTATTACAGTACTGGTGGCACTCAGCTTCAACT			
	GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA			
601	CTGGCTCGTCTTTATAATTGCATCCAAATGATTCAGAAGCAGCAAG			
	GACCGAGCAAGGAAATATTAACGTAGGTTACTAAAGTCTCGTCGTT			
651	ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA			
	TAAGGTTATATAACTCCCTTTACCGTGCTCTTAATCCATGTTGGCCT			
701	GATCTGCACCAAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA			
	CTAGACGTGGTCTAGGATCGCATTATGTGAACCTTATCAACCCCTCT			
751	CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT			
	GAAAGGTGACGTTAAGTTCTCAGATTGGTCTCGGAAACGATCAGGTTA			
801	TCAACTGCAAAGACGTAATGGTCCAAATTCAAGTGTGACGATGTGAGTA			
	AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT			
851	TATTAATCCCTATCATAGCTCATGGTGTATAGATGCGCACCTCCACCA			
	ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACCGGTGGAGGTGGT			
901	TCGTCACAGTTCTGTGTTACAGGCATCGACGGGATATGGTAATGC			
	AGCAGTGTCAAAGACACATAATGTCCTAGCTGCCCTATACCATTACG			

76/254

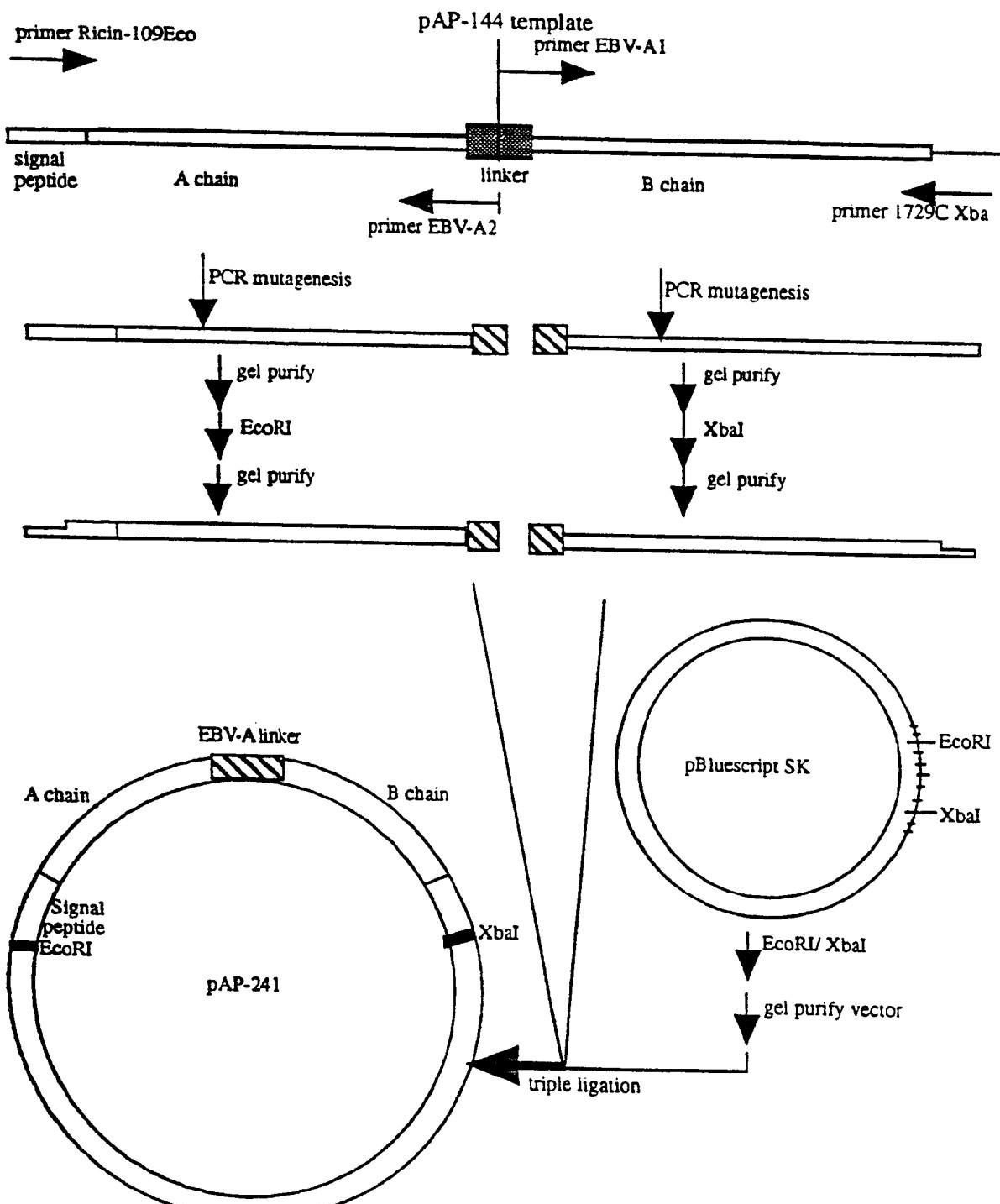
**FIGURE 15D (CONT'D)**

951 TGATGTTGTATGGATCCTGAGCCCATAGTCGTATCGTAGGTCGAAATG  
     ACTACAAACATACTACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
     CAGATACACAACATACTACATCCCTACCTCTAAGGTGTTGCCCTTGCGTTAT  
  
 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
     GTCAACACCCGGTACGTTACAGATTATGTCTACGTTAGTCGAGACCTGAAA  
  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
     CTTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAAAACTGCTGCA  
     CCATGTCAGGCCCTCAGATACACTAGATACTAACGTTATGACGACGT  
  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC  
     TGACTACGGTGGCGACCGTTATACCTTACCTTGTTAGTATTAGG  
  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAAACAGTGGTACCAACAC  
     GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTACCATGGTGTG  
  
 1301 TTACAGTCAAACCAACATTATGCCGTTAGTCAAGGTTGGCTTCTACT  
     AATGTCACGTTGGTTGTAACACGGCAATCAGTTCCAACCGAAGGATGA  
  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG  
     TTATTATGTGTTGGAAAACAATGTTGTAACAAACCGATATACCAGACAC  
  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGAGCTGTAGCAGTGAAA  
     GAACGTTCTGTTATCACCTGTTACACCTATCTCCTGACATCGTCACTTT  
  
 1451 AGGCTGAACAAACAGTGGGCTTTATGCAAGATGGTCAATACGTCCCTCAG  
     TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGATTCTAATATACGGGAAACAGT  
     GTTTGCGCTTAAACGGAATGTTCACTAAGATTATGCCCTTGTC  
  
 1551 TGTTAAGATCCTCTTTGTGGCCCTGCATCCTCTGGCAACGATGGATGT  
     ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA  
  
 1601 TCAAGAATGATGGAACCATTAAATTGTTAGTGGATTGGTTAGAT  
     AGTTCTTACTACCTGGTAAATTAACATATCACCTAACCAACATCTA  
  
 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTACCCCTCTCCA  
     CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
  
 1701 TGGTGACCCAAACCAAAATATGGTTACCATTATTTGATAGACAGATTACT  
     ACCACTGGGTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA  
  
 1751 CTCTTGCACTGTGTCCTGCCATGAAAATAGATGGCTAAATAAAAAA  
     GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATT  
  
 1801 GGACATTGTAATTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC  
     CCTGTAACATTAAAACATTGACTTCTGCTGTTCAATATAGCTTAAAGG  
  
 1851 TGCAG  
     ACGTC

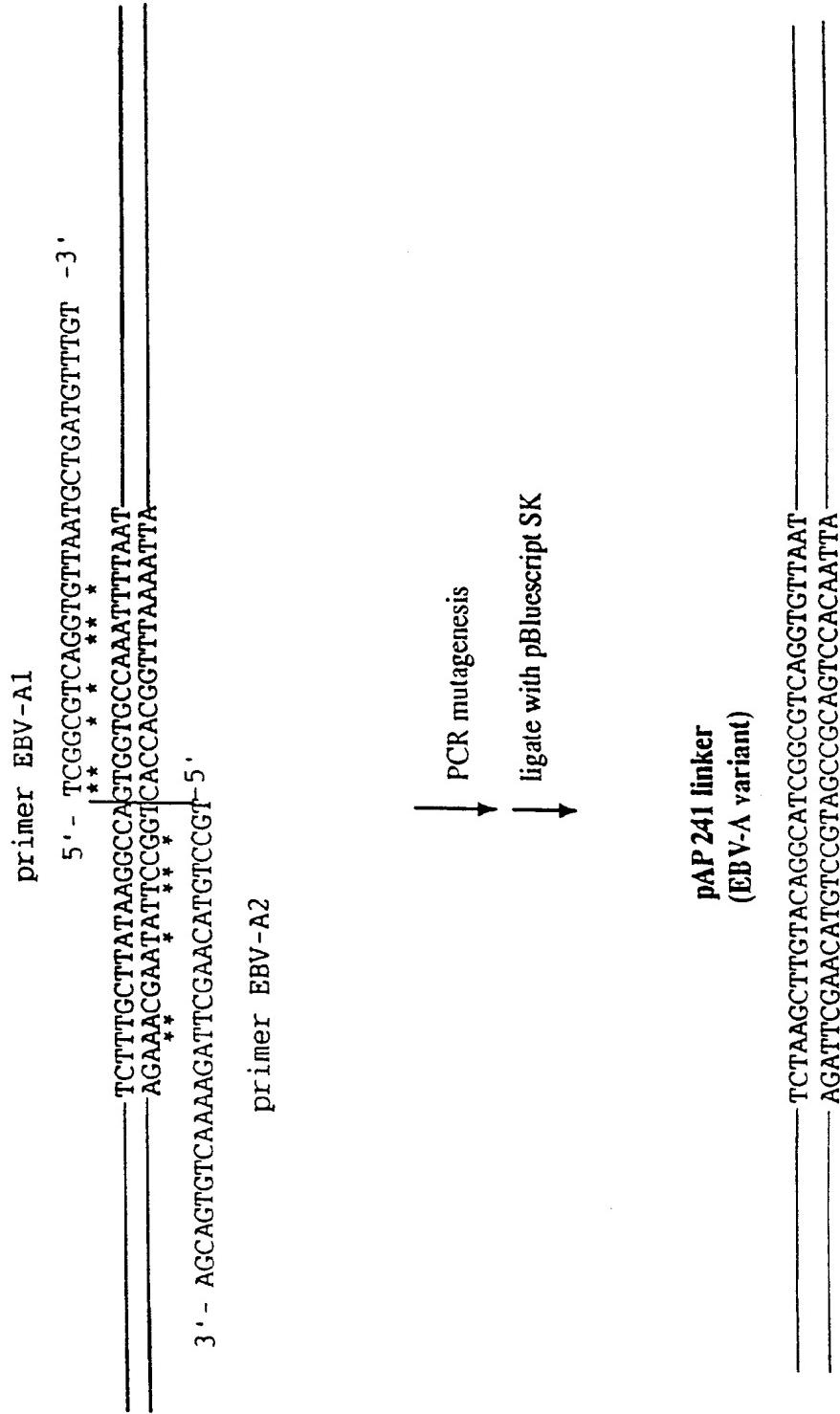
77/254

**FIGURE 16A**

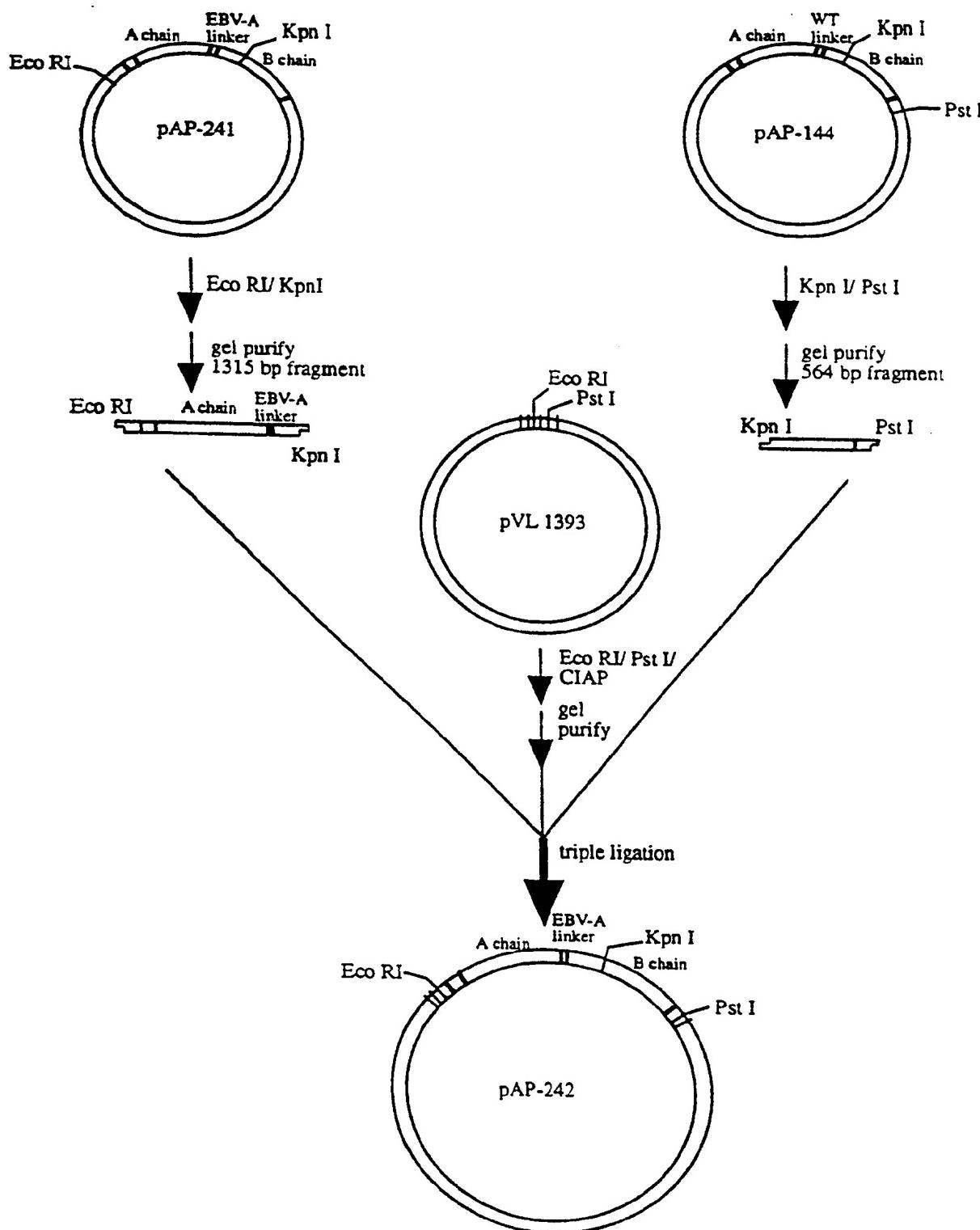
**PCR Mutagenesis of Preproricin Gene to Create an EBV-A Variant  
Gene a) Cloning Strategy**



78/254

**FIGURE 16B****WT preprorocin linker**

79/254

FIGURE 16C

80/254

FIGURE 16D

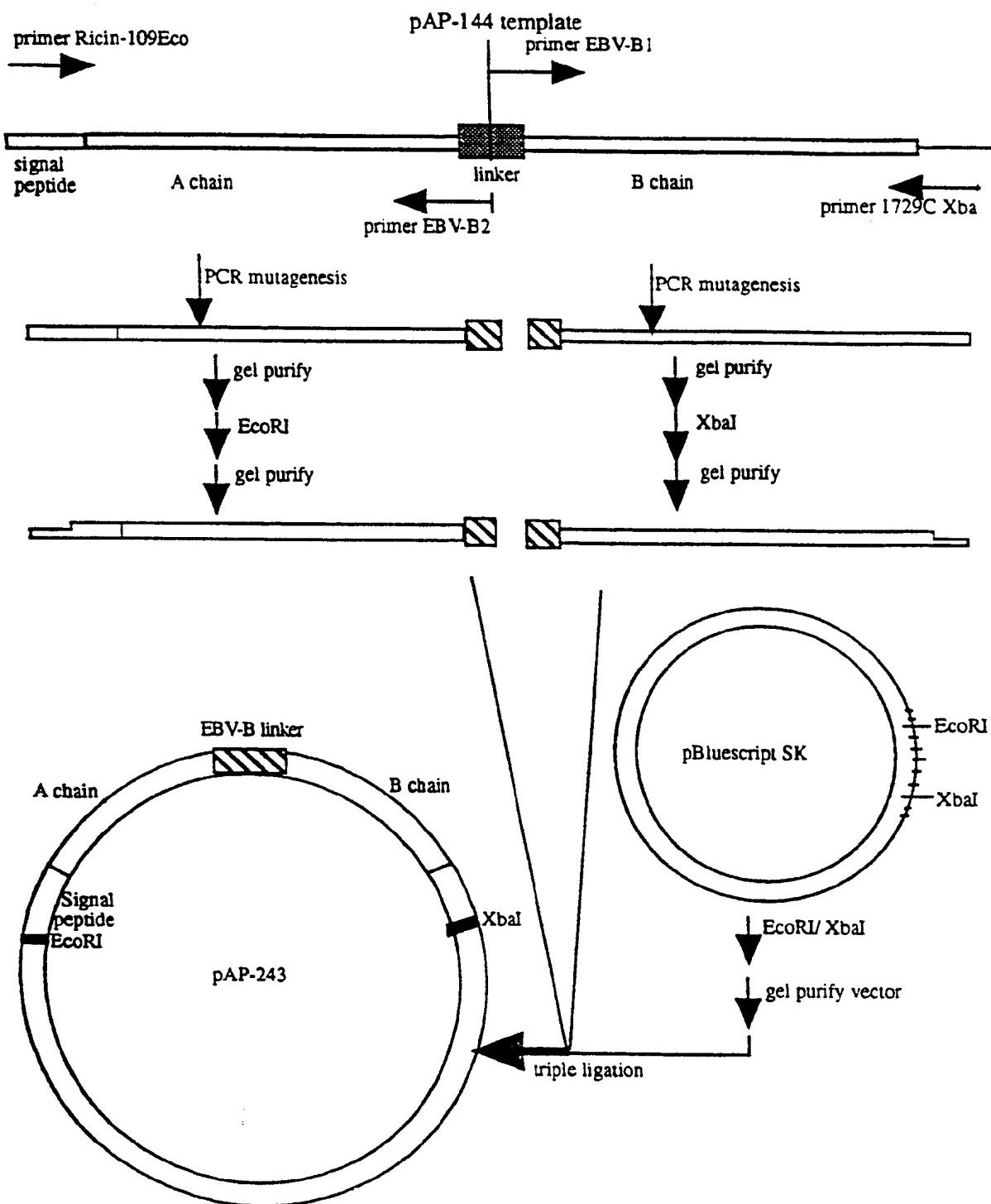
10	20	30	40	50
1	GAATTCATGAAACCGGGAGGAATACTATTGTAAATATGGATGTATGCAGT			
	CTTAAGTACTTTGGCCCTCCTTATGATAACATTACCTACATACGTCA			
51	GGCACACATGGCTTGTGATCCACCTCAGGGTGGCTTCACATTAG			
	CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAA			
101	AGGATAACAACATATTCCCCAACAACTACCAATTATAAATTTACCA			
	TCCTATTGTTGATAAGGGTTGTTATGGTTAATATTGAAATGGT			
151	GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTTCGCGG			
	CGCCCCACGGTGACCGTTGATGTGTTGAAATAGTCTCGACAAGCGCC			
201	TCGTTAACAACTGGAGCTGATGTGAGACATGATATAACCAGTGTGCCAA			
	AGCAAATTGTTGACCTCGACTACACTCTGACTATATGGTCACAACGGTT			
251	ACAGAGTTGGTTGCCTATAAACCAACGGTTATTAGTTAGTTGAAC			
	TCTCAACCAAACGGATATTGGTTGCCAAATAAAACTCAACTTGAGAGT			
301	AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA			
	TTAGTACGTCTCGAAAGACAATGTAATCGCACCTACAGTGGTTACGTAT			
351	TGTGGTCGGCTACCGTGTGGAAATAGCGCATATTCTTCATCCTGACA			
	ACACCAAGCCGATGGCACGACCTTATCGGTATAAAGAAAGTAGGACTGT			
401	ATCAGGAAGATGCAGAAGCAACTCACTCATCTTCACTGATGTTCAA			
	ATAGTCCTTCTACGTCTTGTAGTAGAAAGTAGACTACAAGTTA			
451	CGATATACTCGCTTGGTGGTAATTATGATAGACTTGAACAAC			
	TGCTATATGTAAGCGGAAACCACCAATTAAACTATCTGAAC			
501	TGGTAATCTGAGAGAAAAATATCGAGTTGGAAATGGTCACTAGAGGAGG			
	ACCATTAGACTCTTTATAGCTCAACCCCTTACCAAGGTGATCTCCTCC			
551	CTATCTCAGCGCTTATTACAGTACTGGGGCACTCAGCTTCAA			
	CTGATGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA			
601	CTGGCTCGTTCTTATAATTGCACTCAAATGATTCAAGCAGCAAG			
	GACCGAGCAAGGAAATATAACGTAGGTTACTAAAGTCTCGTCGTT			
651	ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA			
	TAAGTTATATAACTCCCTTTACCGCGTCTTAATCCATGTTGGCCT			
701	GATCTGCAACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA			
	CTAGACGTGGCTAGGATCGCATTATGTGAACCTTATCAACCCCTCT			
751	CTTTCACGTCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCAA			
	GAAAGGTGACGTTAAGTCTCAGATTGGTCTCGGAAACGATCAGGTTA			
801	TCAACTGCAAAGACGTAATGGTCAAATTCACTGAGTGTACGATGTGAGTA			
	AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT			
851	TATTAATCCCTATCATAGCTCTCATGGTGTAGATGCGCACCTCCACCA			
	ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACCGCGTGGAGGTGGT			
901	TCGTCACAGTTCTAACGCTGTACAGGCATCGCGTCAGGTGTTAATGC			
	AGCAGTGTCAAAAGATTGAAACATGTCGCTAGCCGAGTCCACAAATTACG			

81/254

**FIGURE 16D (CONT'D)**

951 TGATGTTGTATGGATCCTGAGCCCATACTGCGTATCGTAGGTCGAAATG  
 ACTACAAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
 CAGATACACAACATACTACATCCCTACCTCTAAGGTGTTGCCTTGCGTTAT  
  
 1051 CAGTTGTGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCCTGACGTTACGATTATGTCTACGTTAGTCGAGACCTGAAA  
  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
  
 1151 GGTACAGTCGGGAGTCTATGTGATGATCTATGATTGCAAACTGCTGCA  
 CCATGTCAGGCCCTCAGATAACACTAGATAACTAACGTTATGACGACGT  
  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC  
 TGACTACGGTGGCGACCCTTATACCCCTATTACCTTGGTAGTTAGG  
  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
 GTCTAGATCAGATAAAATCGTCGCTGTAGTCCCTGTCACCATGGTGTG  
  
 1301 TTACAGTGCACCAACATTTATGCCCTTAGTCAGGTTGGCTTCTACT  
 AATGTCACGTTGGTTGTAATACGGCAATCAGTCCAACCGAAGGATGA  
  
 1351 AATAATACACAAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
 TTATTATGTGTTGAAAACAATGTTGTAACAACCCGATATACCAAGACAC  
  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTAAA  
 GAACGTTGTTTATCACCTGTTACACCTATCTCCTGACATCGTCACTTT  
  
 1451 AGGCTGAACAAACAGTGGCCTTTATGCAGATGGTTCAATACGTCTCAG  
 TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGATTCTAATATAACGGGAAACAGT  
 GTTTGGCTCTATTAACGGAATGTTACTAAGATTATATGCCCTTGTC  
  
 1551 TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCAACGATGGATGT  
 ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA  
  
 1601 TCAAGAATGATGGAACCATTAAATTGTATAGTGGATTGGTGTAGAT  
 AGTTCTTACTACCTGGTAAAATTAAACATATCACCTAACCAACATCTA  
  
 1651 GTGAGGCAGTCGGATCCGAGCCTTAAACAAATCATTCTTACCCCTCTCCA  
 CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
  
 1701 TGGTGAACCAAACCAAATATGGTTACCATTATTTGATAGACAGATTACT  
 ACCACTGGGTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA  
  
 1751 CTCTTGCAGTGTGTTGCTGCCATGAAAATAGATGGCTAAATAAAAAA  
 GAGAACGTCACACACAGGACGGTACTTTATCTACCGAATTATTATTTT  
  
 1801 GGACATTGTAATTGGTAACTGAAAGGACAGCAAGTTATATCGAATTCC  
 CCTGTAACATTAAAACATTGACTTCCCTGTCGTTCAATATAGCTTAAGG  
  
 1851 TGCAG  
 ACGTC

82/254

FIGURE 17A

83 / 254

## FIGURE 17B

WT preprorocin linker

primer EBV-B2

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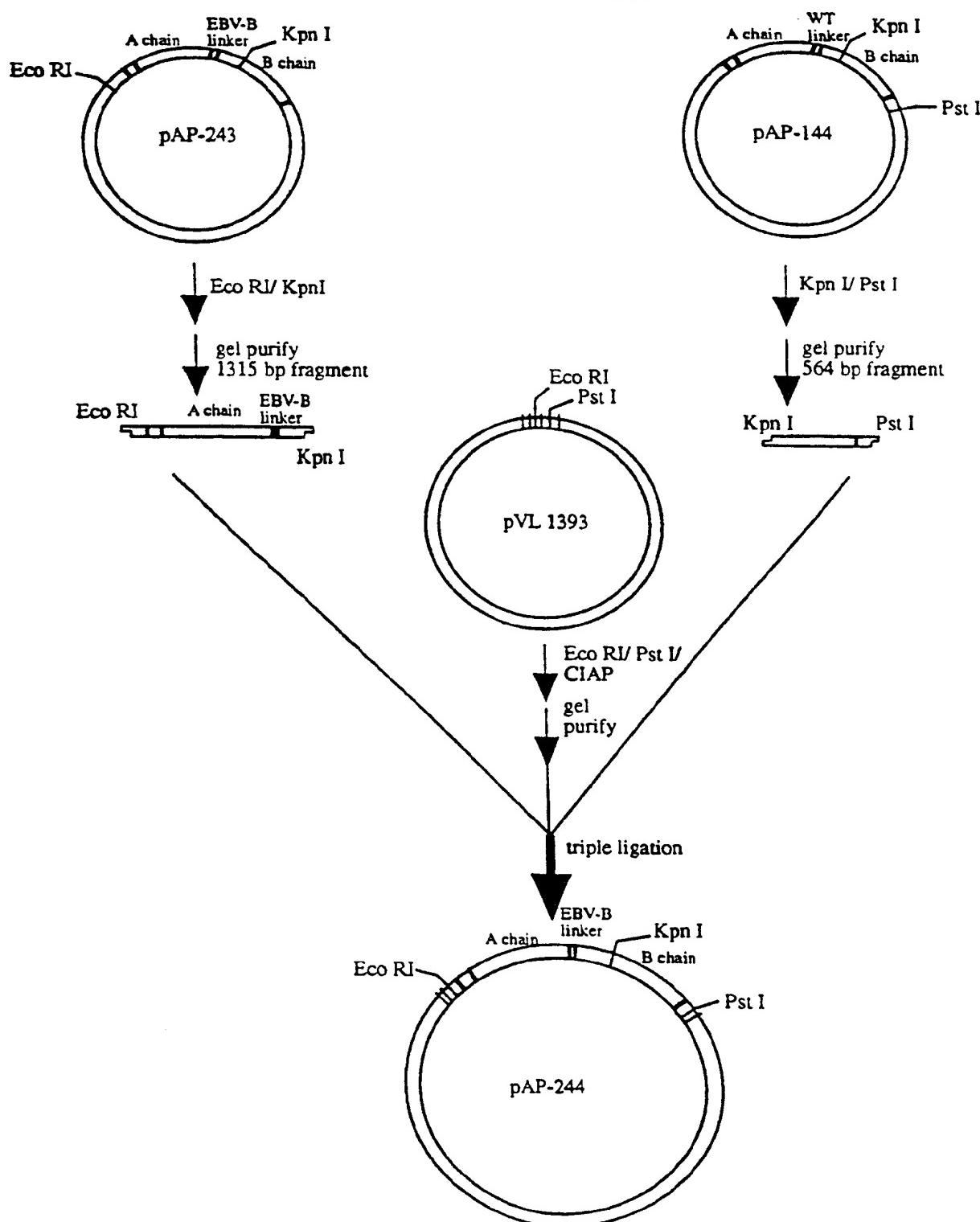
graph TD
    A[PCR mutagenesis] --> B[ligate with pBluescript SK]

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pAP243 linker  
(EBV-B variant)

TCTTCGTTATCTAAAGGCATCGGACGCCCTGATAAT  
AGAAGGCATAGATTCCGTAGCCTGCGTGGACTATTA

84/254

FIGURE 17C

85/254

FIGURE 17D

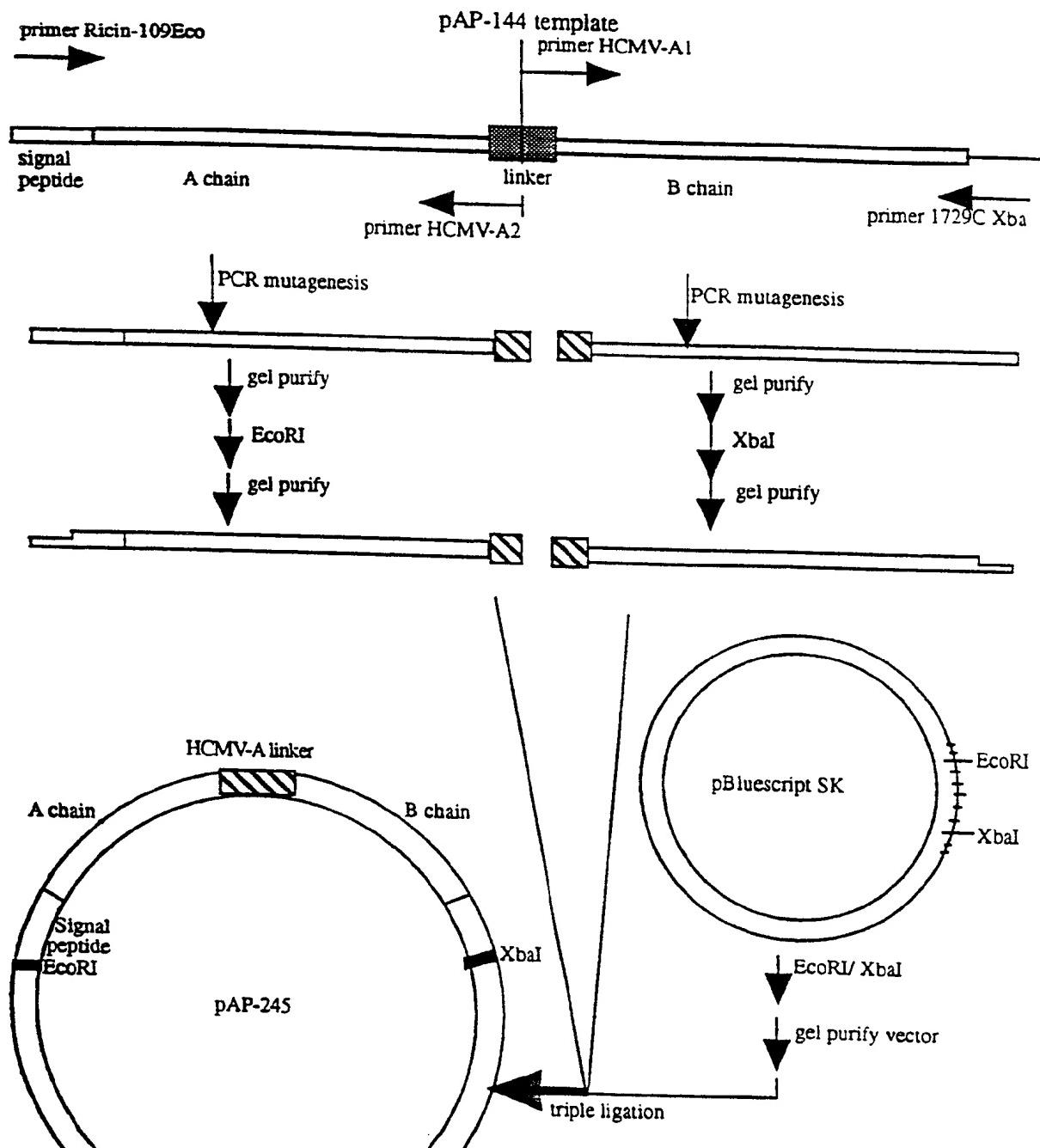
10	20	30	40	50
1 GAATT	CATGAA	ACCGGGAGG	AAATACTATT	GTAATATGGATGTATGCAGT
	TTAAGTACTT	TGGCCCTCCTT	TATGATAACATT	TACCTACATACGTCA
51 GGCAACATGG	CTTGTGATCCAC	CTCAGGGTGG	TTCACATTAG	
	CGTGTACCGAA	ACAAACCTAG	GGAGTCCCACCAGAA	AGTGTAA
101 AGGATAACA	ACATATT	CCCCAAACA	ATACCCAA	TTAACCTTACCA
	TCCTATTGTT	GATAAGGG	TTGTATGG	TTAATATTGAAATGGT
151 GCGGGTGCC	ACTGTGCAA	AGCTACACAA	ACCTTATCAGAGCTG	TTCGCC
	CGCCACGGTGAC	ACGTTCGATGT	GGTAAATAGTCTCG	ACAAGCGCC
201 TCGTTAACAA	ACTGGAGCTG	ATGTGAGACATG	ATACCA	AGTGTGCCAA
	AGCAAATTGTT	GACCTCGACTAC	ACTCTGTACT	ATATGGTCACAACGGTT
251 ACAGAGTTGG	TTGCCTATAA	ACCAACGG	TTATTTAG	TTGAAC
	TGTCTCAACCA	ACGGATATTG	GGTGC	AAATAAAACTCAACTTGAGAGT
301 AATCATGCAGAG	CTTCTGTTAC	ATTAGCGCTGG	ATGTCA	CCAATGCATA
	TTAGTACGTCTG	AAAGACA	ATGTA	ATCGCACCTACAGTGGTTACGT
351 TGTGGTCGG	CTACCGTGTG	GGAAATAGCGC	ATATTCTTCAT	CCGACA
	ACACCAGCCGAT	GGCACG	ACCTTATCGCGT	ATAAGAAAGTAGGACTGT
401 ATCAGGAAGAT	GCAGAAC	CAATCACTC	ATCTTTCA	CTGATGTTAAAAT
	TAGTCCTCTAC	GTCTCG	TTAGTGA	AAAGTGA
451 CGATATACAT	CGCCTTGGT	GGTAATTATG	ATAGACTTGA	ACAAACTTGC
	GCTATATGTAAG	CGGAAACCAC	ATTAA	ACTATCTGAAC
501 TGGTAATCTGAGAG	AAAAATATGAG	TTGGGAAATGGT	CCACTAGAGG	AGG
	ACCATTAGACT	CTCTTTATAG	CTAACCC	TTTACCA
551 CTATCTCAGCG	CTTTATTATTAC	AGTACTGGT	GGCACTCAGCT	CCAACT
	GATAGAGTC	CGAAATAA	ATATGT	CATGACCACCGTGAGTC
601 CTGGCTCG	TCTCTTTATAATTG	CATCCA	ATGATTTCAGA	AGCAGCAAG
	GACCGAGCA	AGGAAATATTAA	ACGTAGGTT	ACTAAAGTCTCGT
651 ATTCCAATAT	ATTGAGGGAG	AAATGCGCAC	GAGAATTAGGT	ACAACCGGA
	TAAGGTT	TATAACT	CCCTCTTAC	CGTCTTAATCC
701 GACTGCA	CCAGATCCTAG	CGTAATTAC	ACTTGA	GAATAGTTGGGGAGA
	CTAGACGTGG	TCTAGGAT	CGCATTAA	GTGAAC
751 CTTCCACTGCA	ATTCAAGAGT	CTAACCAAGGAG	GCCTTGCTAG	TCATG
	GAAAGGTGAC	TTAAGTCTCAG	ATTGGT	CCCTCGGAAACGATCAGGTTA
801 TCAACTGCA	AAAGACGTA	ATGGTCAA	ATTCACTG	GATGTGAGTA
	AGTTGACGTTCTG	CATTACCA	AGGTTAAGT	CACACATGCTACACTCAT
851 TATTAATCC	TATCATA	AGCTCTCATGGT	TATAGATGCGC	ACCTCCACCA
	ATAATTAGGG	ATAGTATCG	AGAGTACCA	CATATCTACGCGTGGAGGTGG
901 TCGTCACAG	TTTCTCGT	ATCTAAAGG	CATCGGACG	CACCTGATAATGC
	AGCAGTGT	CAAAGAAGCA	TAGATTTCCG	TAGCCTCGGTGGACTATTACG

86/254

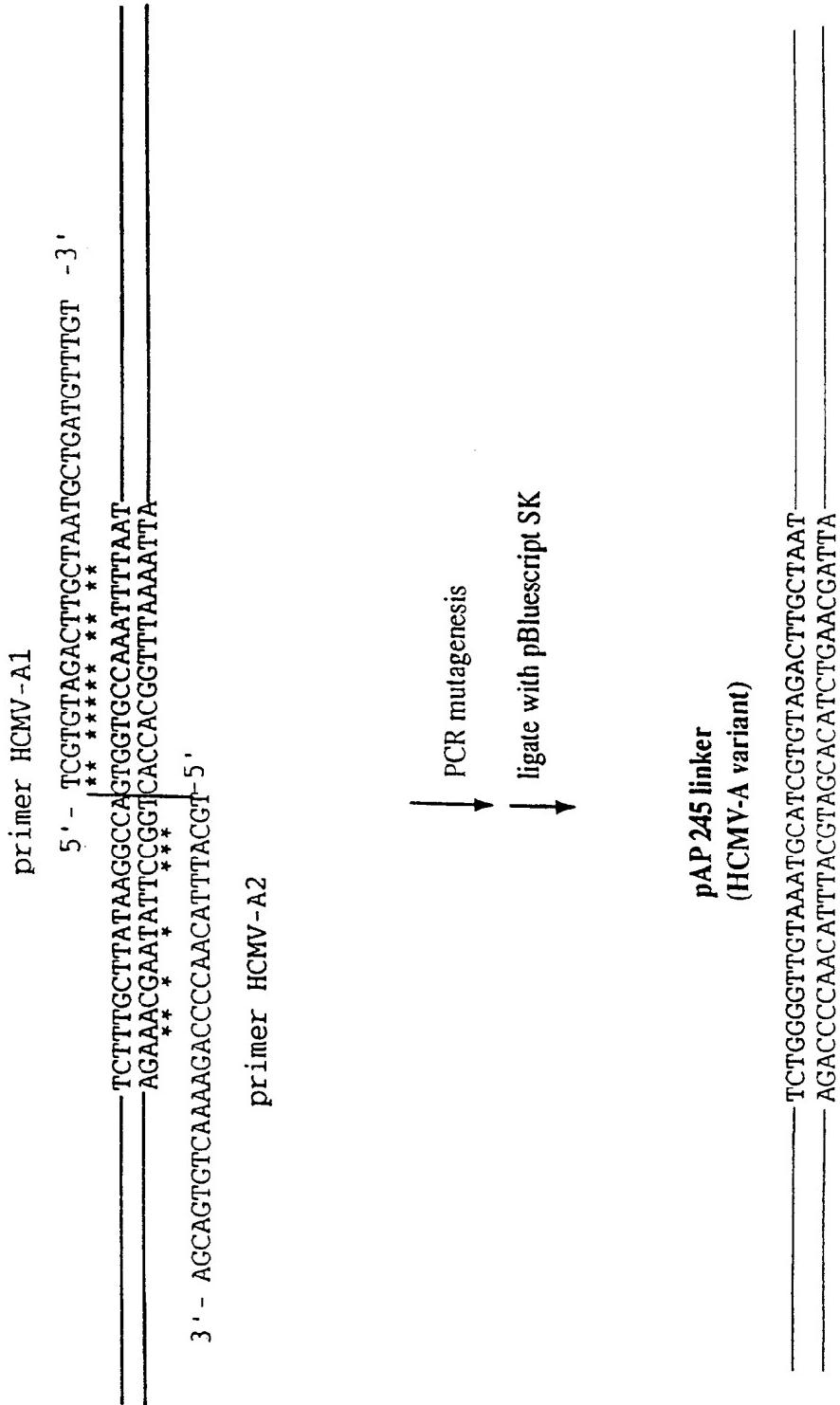
**FIGURE 17D (CONT'D)**

951 TGATGTTGTATGGATCCTGAGCCCATA GTGCGTATCGTAGGTCGAAATG  
 ACTACAAACATA CCTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GCTATGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
 CAGATACACAAC TACAATCCCTACCTTCAAGGTGTTGCCTTGCGTTAT  
 1051 CAGTTGTGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCAGTACGTTCA GATTATGTC TAGCTTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTAACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA  
 CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACC ATCATAAATCC  
 TGACTACGGTGGCGACCGTTATACCTTACCTTACCTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
 GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTCACCATGGTGTG  
 1301 TTACAGTGCAAACCAACATTATGCCGTTAGTCAAGGTGGCTCCTACT  
 AATGTCACGTTGGTTGTAATAACGGCAATCAGTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
 TTATTATGTTGAAAACATGTTGTAACAACCGATATAACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
 GAACGTTCGTTATCACCTGTTCAACCTATCTCCTGACATCGTCACTT  
 1451 AGGCTGAACAACAGTGGCTCTTATGCAGATGGTCAATACGTCCTCAG  
 TCCGACTTGTGTCACCCGAGAAAATACGTCTACCAAGTTATGCAAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGATTCTAATATACGGGAAACAGT  
 GTTTGGCTCTATTAAACGGAATGTTCACTAAGATTATATGCCCTTGTC  
 1551 TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
 ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA  
 1601 TCAAGAATGATGGAACCATTAAATTGTTAGTGGATTGGTGTAGAT  
 AGTTCTTACTACCTTGGTAAATTTAAACATATCACCTAACCAATCTA  
 1651 GTGAGGCGATCGGATCCGAGCCTAAACAAATCATTCTTACCCCTCTCCA  
 CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGAGAGGT  
 1701 TGGTGACCCAAACCAAATATGGTTACCAATTATTGATAGACAGATTACT  
 ACCACTGGGTTGGTTATACCAATGGAATAAAACTATCTGTCTAATGA  
 1751 CTCTTGCA GTGTTGTCCTGCCATGAAAATAGATGGCTAAATAAAA  
 GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATTTT  
 1801 GGACATTGTAATTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC  
 CCTGTAACATTAAAACATTGACTTTCTGTCGTTCAATATAGCTTAAGG  
 1851 TGCAG  
 ACGTC

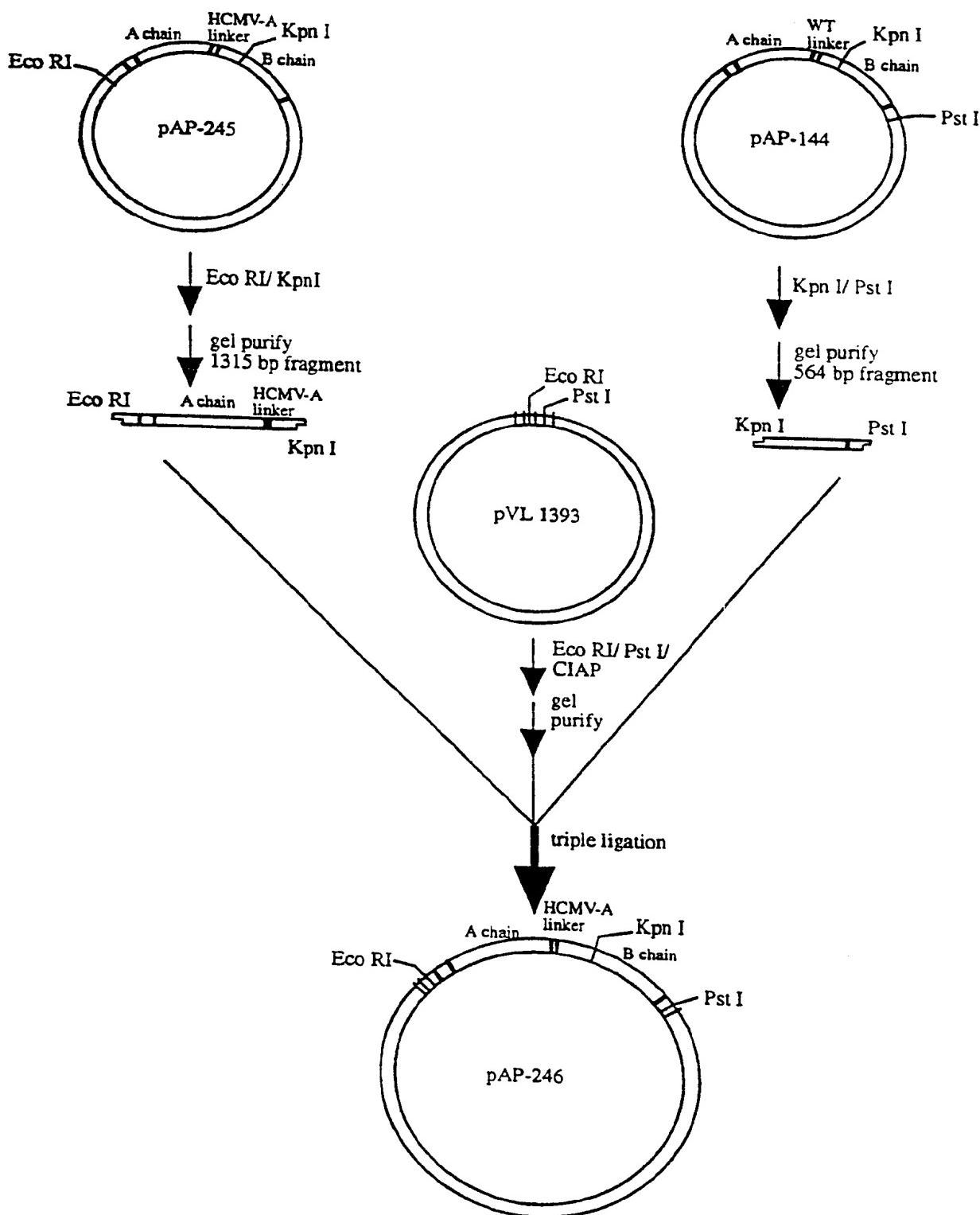
87/254

**FIGURE 18A**

88/254

**FIGURE 18B****WT preprorin linker**

89/254

FIGURE 18C

90/254

**FIGURE 18D**

1	10	20	30	40	50
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1  GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
   CTTAAGTACTTTGGCCCTCCTTATGATAACATTACCTACATACGTCA

51  GGCAACATGGCTTGTGATCCACCTCAGGGTGGCTTTCACATTAG
   CGGTTGTACCGAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAA

101 AGGATAACAACATATTCCCCAACAACTCCAATTATAAACTTACCA
    TCCTATTGTTGTATAAGGGTTGTTATGGGTTAATATTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTTATCAGAGCTGTTCGCGG
    CGCCCACGGTGACACGTTGATGTGTTGAAATAGTCTCGACAAGCGCC

201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCAAGTGGTCCAA
    AGCAAATTGTTGACCTCGACTACACTCTGACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTGCCCTATAAACCAACGGTTTTAGTTAGTTGAACCTCTCA
    TGTCTCAACCAACGGATATTGGTTGCCAAATAAAACTCAACTTGAGAGT

301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
    TTAGTACGTCTCGAAAGACAATGTAATCGCAGCTACAGTGGTACGTAT

351 TGTGGTCGGCTACCGTGGAAATAGCGCATATTCTTACATCGCTGACA
    ACACCAGCCGATGGCACGACCTTATCGCTGATAAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCGAGAACATCACTCATCTTCACTGATGTTCAAAT
    TAGTCCTCTACGTCTCGTTAGTGAGTAGAAAGTGAACACTAAGTTTA

451 CGATATACATTGCCCTTGGTGGTAATTATGATAGACTTGAAACAACCTGC
    GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACCTGTTGAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG
    ACCATTAGACTCTTTATAGCTAACCCCTTACAGGTGATCTCCTCC

551 CTATCTAGCGCTTATTACAGTACTGGTGGCACTCAGCTTCAACT
    GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTTCTTATAATTGCACTCCAAATGATTCAGAAGCAGCAAG
    GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCCTCGTCGTT

651 ATTCCAATATATTGAGGGAGAAAATGCGCACGAGAATTAGGTACAACCGGA
    TAAGGTTATATAACTCCCTTTACCGTGTCTTAATCCATGTTGGCCT

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA
    CTAGACGTGGTCTAGGATCGATTAAATGTAACCTTATCAACCCCTCT

751 CTTTCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT
    GAAAGGTGACGTTAAGTCTCAGATTGGTCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTCCAAATTCACTGATGTGAGTA
    AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAAGATGCGCACCTCCACCA
    ATAATTAGGGATAGTACGAGAGTACCAACATCTACCGTGGAGGTGGT

901 TCGTCACAGTTCTGGGGTTGTAATGCATCGTAGACTTGCTAATGC
    AGCAGTGTCAAAAGACCCCAACATTACGTAGCACATCTGAACGATTACG

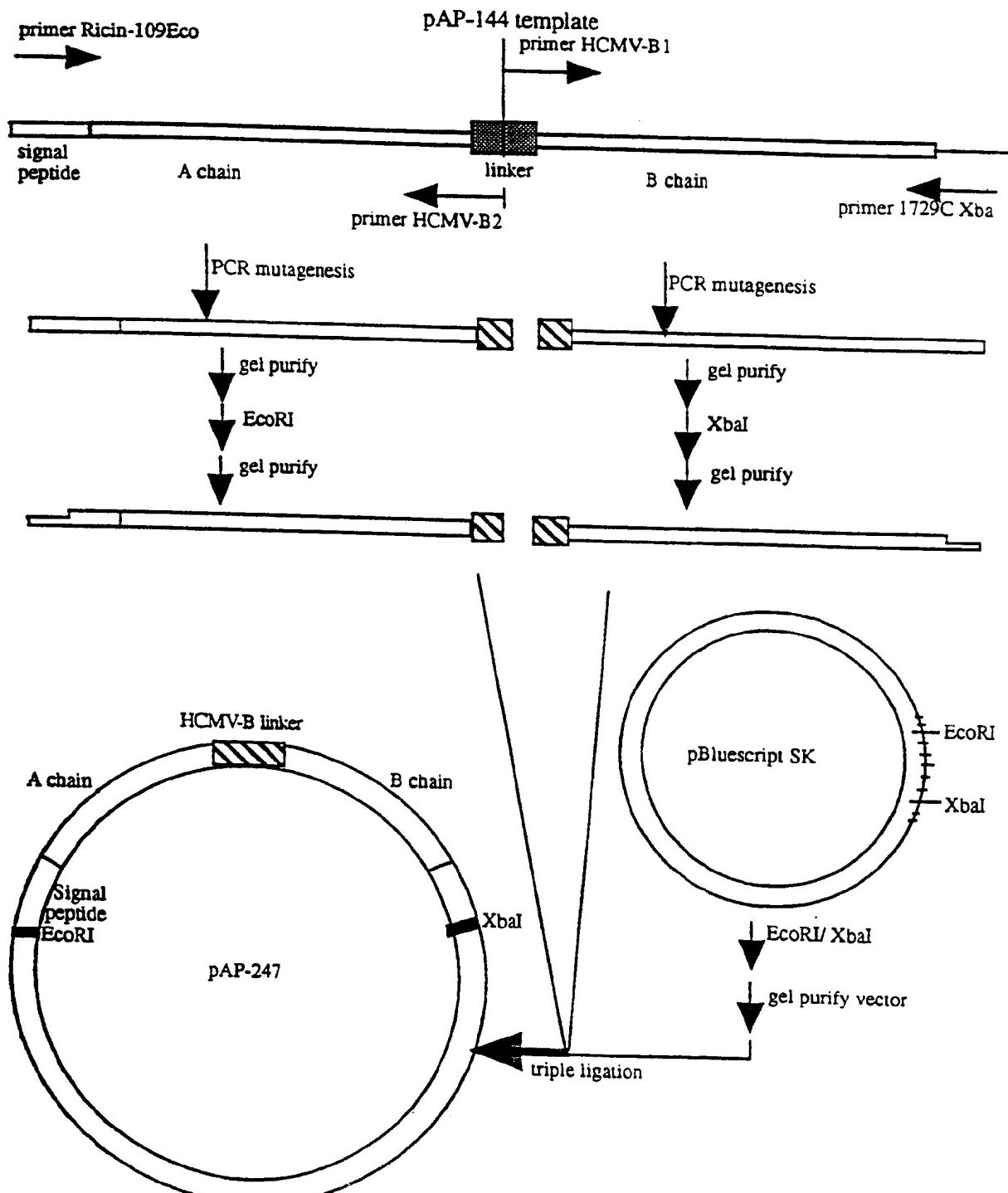
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91/254

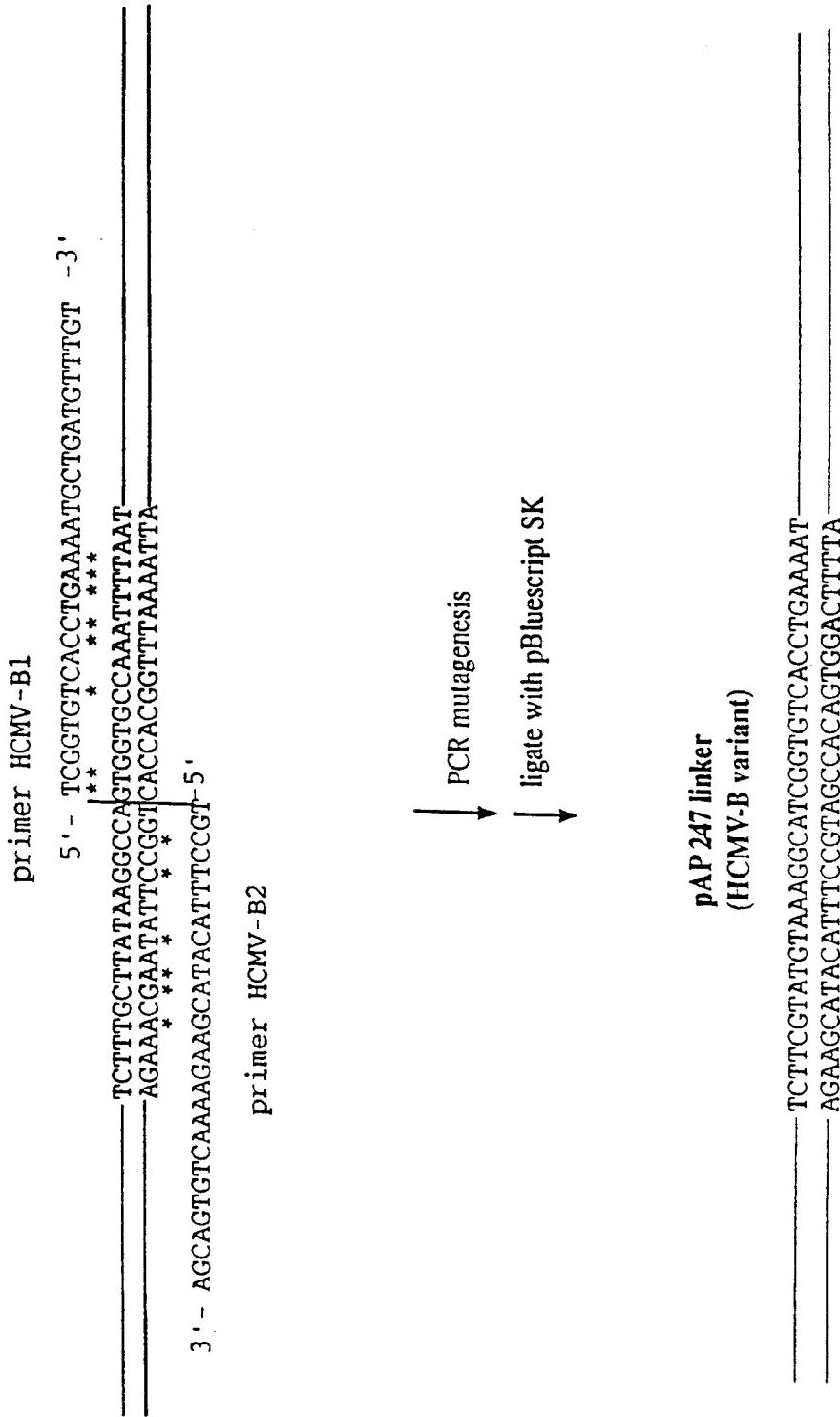
**FIGURE 18D (CONT'D)**

951 TGATTTGTATGGATCCTGAGCCATAGTCGTATCGTAGGTCGAAATG  
     ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
     CAGATACACAACATAATCCCTACCTCTAAGGTGTTGCTTGCCTTGCGTTAT  
 1051 CAGTTGTGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
     GTCAACACCGGTACGTTAGATTATGTCTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
     CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA  
     CCATGTCAGGCCCTCAGATACACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAATCC  
     TGACTACGGTGGCGACCGTTATACCTTACCTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGAAACAGTGGTACCAACAC  
     GTCTAGATCAGATCAAATCGTCGCTGAGTCCCTGTCACCATGGTGTG  
 1301 TTACAGTGCACCAACATTATGCCGTTAGTCAGGTTGGCTTCACT  
     AATGTCACGTTGGTGTAAATACGGCAATCAGTCCAACCGAAGGATGA  
 1351 AATAATACACACACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
     TTATTATGTGTTGGAAAACATGTTGTAACAACCCGATATACCAAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
     GAACGTTCGTTATCACCTGTTACACCTATCTCTGACATCGTCACTTT  
 1451 AGGCTGAACAAACAGTGGCTTTATGCAGATGTTCAATACGTCCTCAG  
     TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
 1501 CAAAAACCGAGATAATTGCCCTACAAGTGATTCTAATATACGGAAACAGT  
     GTTTTGGCTCTATTAACGGAAATGTTCACTAAGATTATATGCCCTTGTC  
 1551 TGTTAAGATCCTCTTGTTGGCCCTGCATCCTCTGGCAACGATGGATGT  
     ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA  
 1601 TCAAGAATGATGGAACCATTAAATTGTTAGTGATAGGGATTGGTGTAGAT  
     AGTTCTTACTACCTGGTAAATTAACATATCACCTAACCAACAACTCTA  
 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTACCCCTCCA  
     CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
 1701 TGGTGACCCAAACCAAATATGGTACCATTTGTTAGAGACAGATTACT  
     ACCACTGGGTTGGTTATACCAATGTAATAAAACTATCTGTCTAATGA  
 1751 CTCTTGCACTGTGTTGCTGCCATGAAAATAGATGGCTAAATAAAAAA  
     GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAACATTATT  
 1801 GGACATTGTAATTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC  
     CCTGTAACATTAAAACATTGACTTTCTGTCGTTCAATAAGCTTAAGG  
 1851 TGCAG  
     ACGTC

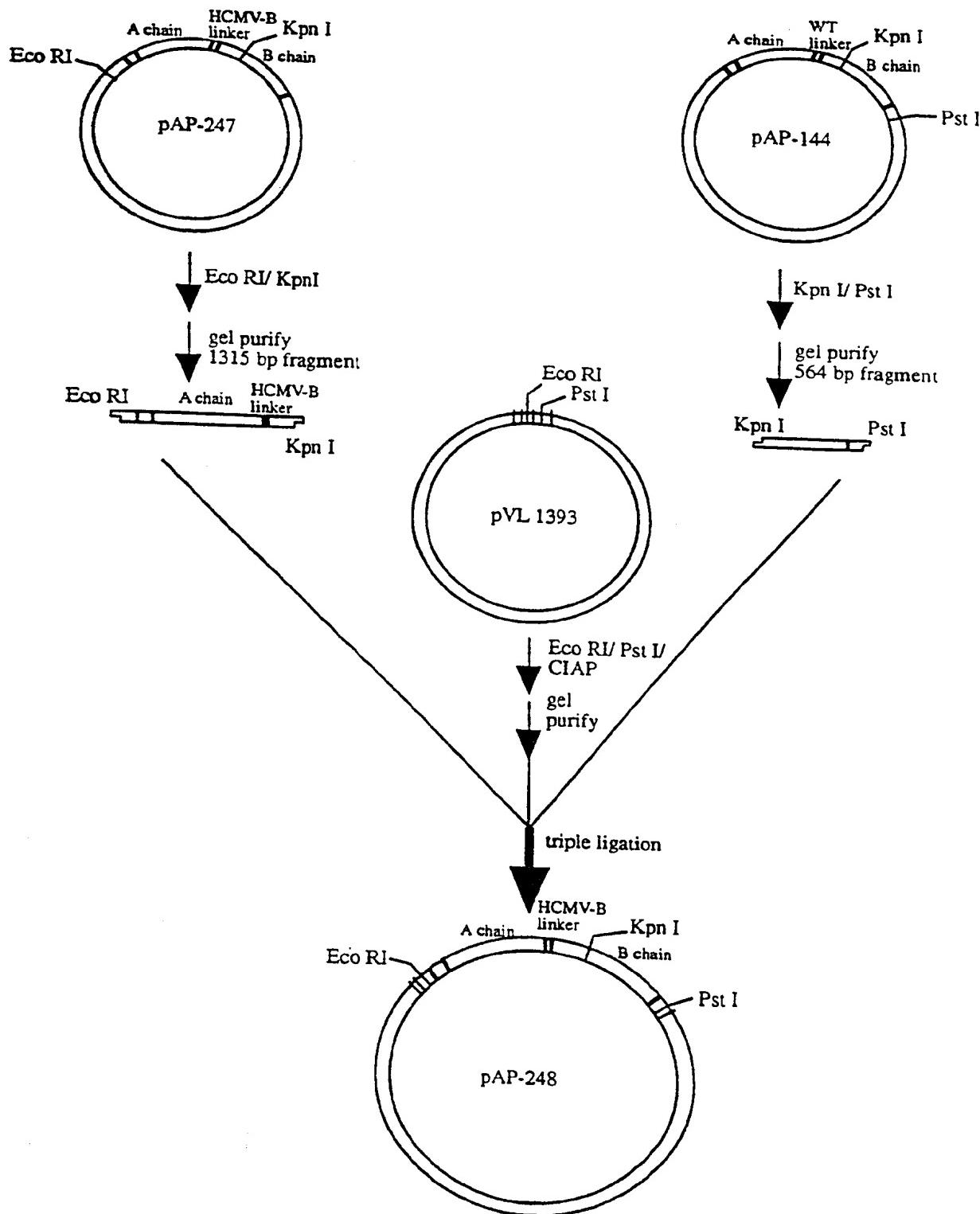
92/254

**FIGURE 19A**

93/254

**FIGURE 19B****WT preprotecin linker**

94/254

FIGURE 19C

95/254

FIGURE 19D

10            20            30            40            50

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1  GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
   CTTAAGTACTTGGCCCTCCTTATGATAACATTATACCTACATACGTCA

51  GGCAACATGGCTTGTTGGATCCACCTCAGGGTGGCTTACATTAG
   CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC

101 AGGATAACAAACATATTCCCCAAACAAATACCCAAATTATAAACCTTACCCACA
   TCCTATTGTTGATAAGGGTTTGTATGGTTAATATTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTTCGCGG
   CGCCCACGGTACACGTTGATGTGTTGAAATAGTCTGACAAAGCGCC

201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCAAGTGTGCCAA
   AGCAAATTGTTGACCTCGACTACACTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTGCCCTATAAACCAACGGTTTTAGTTAGTTGAACCTCTCA
   TGTCCTAACCAAACGGATATTGGTTGCCAAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTCGTTACATTAGCGCTGGATGTCACCAATGCATA
   TTAGTACGTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGTGGAAATAGCGCATATTCTTCATCCTGACA
   ACACCAGCCGATGGCACGACCTTATCGCGTATAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCGAGAAGCAATCACTCATCTTCACTGATGTTAAAAT
   TAGTCCTCTACGTCTCGTTAGTGTAGAAAAGTGAACACTAAGTTTA

451 CGATATACATCGCTTGTTGGTAATTATGATAGACTTGAACAACTTGC
   GCTATATGTAAGCGGAAACCAACCTAAACTATCTGAACCTGGTGAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGGAGG
   ACCATTAGACTCTTTATAGCTCAACCCTTACCAAGGTGATCTCCTCC

551 CTATCTCAGCGTTTATTACAGTACTGGTGGCACTCAGCTTCAACT
   GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTTCTTATAATTGCATCCAAATGATTCAGAACGCAGCAAG
   GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTCGTTTC

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
   TAAGGTTATATAACTCCCTTTACGCGTGTCTTAATCCATGTTGGCCT

701 GATCTGCACCAAGATCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA
   CTAGACGTGGTCTAGGATCGCATTAAATGTGAACCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT
   GAAAGGTGACGTTAAGTCTCAGATTGGTCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTCCAAATTCACTGATGTGAGTA
   AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
   ATAATTAGGGATAGTATCGAGAGTACCCACATATCTACGGTGGAGGTGGT

901 TCGTCACAGTTTCTCGTATGAAAGGCATCGGTGTCACCTGAAAATGC
   AGCAGTGTCAAAAGAACATACATTCCGTAGCCACAGTGGACTTTACG

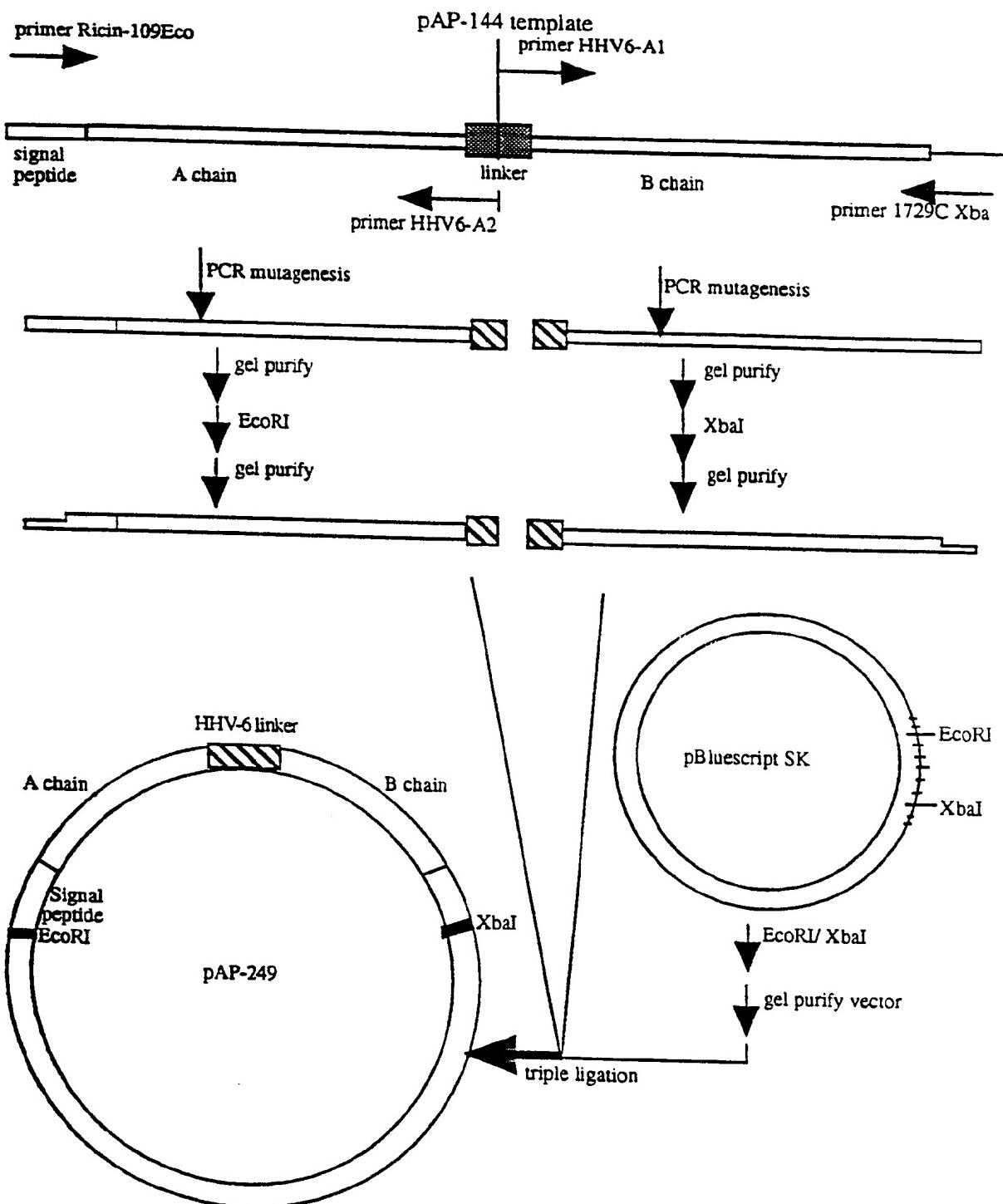
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96/254

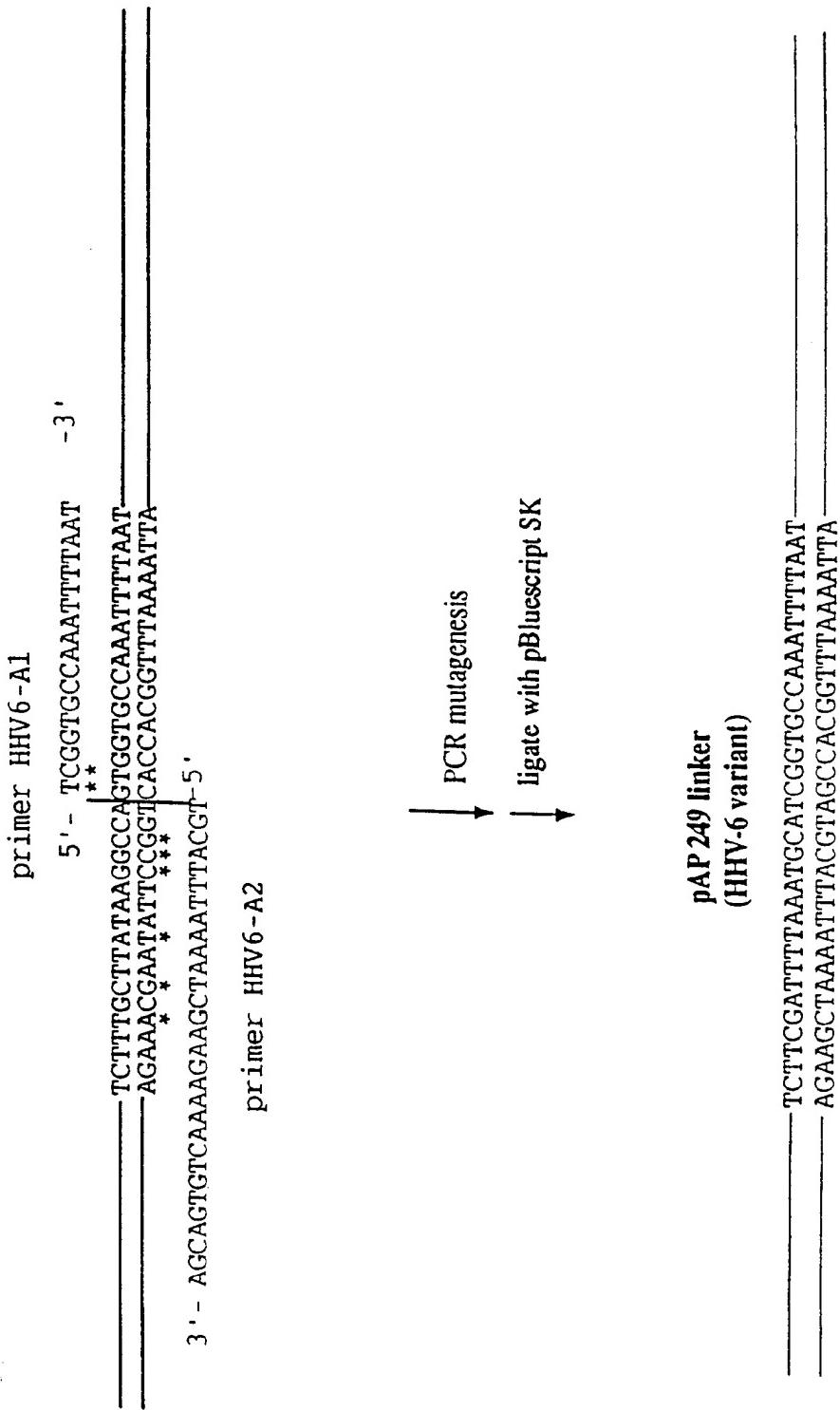
**FIGURE 19D (CONT'D)**

951 TGATGTTGTATGGATCCTGAGCCCATAGTCGTATCGTAGGTCGAAATG  
 ACTACAAACATACTACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
 CAGATACACAACATACTACCTACCTCTAAGGTGTTGCCTTGCGTTAT  
 1051 CAGTTGTGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTAGATTATGCTCTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA  
 CCATGTCAGGCCCTCAGATAACACTAGATAACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCACATCATAAATCC  
 TGACTACGGTGGCGACCGTTATACCTTATTACCTTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC  
 GTCTAGATCAGATAAAATCGTCGCTGTAGTCCCTGTCACCATGGTGTG  
 1301 TTACAGTGCAAACCAACATTATGCCGTTAGTCAGGTTGGCTCCTACT  
 AATGTCACGTTGGTTGTAATAACGGCAATCAGTCCAACCGAAGGATGA  
 1351 AATAATACACAAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
 TTATTATGTGTTGGAAAACAATGTTGTAACAACCCGATATAACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
 GAACGTTGCTTATCACCTGTTACACCTATCTCCTGACATCGTCACCTT  
 1451 AGGCTGAAACAACAGTGGCTCTTATGCAAGATGGTCAATACTCCTCAG  
 TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGATTCTAATATACGGGAAACAGT  
 GTTTGGCTCTATAACGGAAATGTTCACTAAGATTATATGCCCTTGTCA  
 1551 TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
 ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA  
 1601 TCAAGAATGATGGAACCATTAAATTGTATAGTGGATTGGTGTAGAT  
 AGTTCTTACACCTGGTAAATTAAACATATCACCTAACCAATCTA  
 1651 GTGAGGGGATCGGATCCGAGCCTAAACAAATCATTCTTACCCCTCTCCA  
 CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
 1701 TGGTGACCCAAACCAAATATGGTACCAATTATTGATAGACAGATTACT  
 ACCACTGGGTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA  
 1751 CTCTTGCACTGTGTGTCCCTGCCATGAAAATAGATGGCTAAATAAAA  
 GAGAACGTACACACACAGGACGGTACTTTATCTACCGAATTATTTT  
 1801 GGACATTGTAATTGTAACTGAAAGGACAGCAACTTATATCGAATTCC  
 CCTGTAACATTAAACATTGACTTCCCTGTCGTTCAATATAGCTTAAGG  
 1851 TGCAG  
 ACGTC

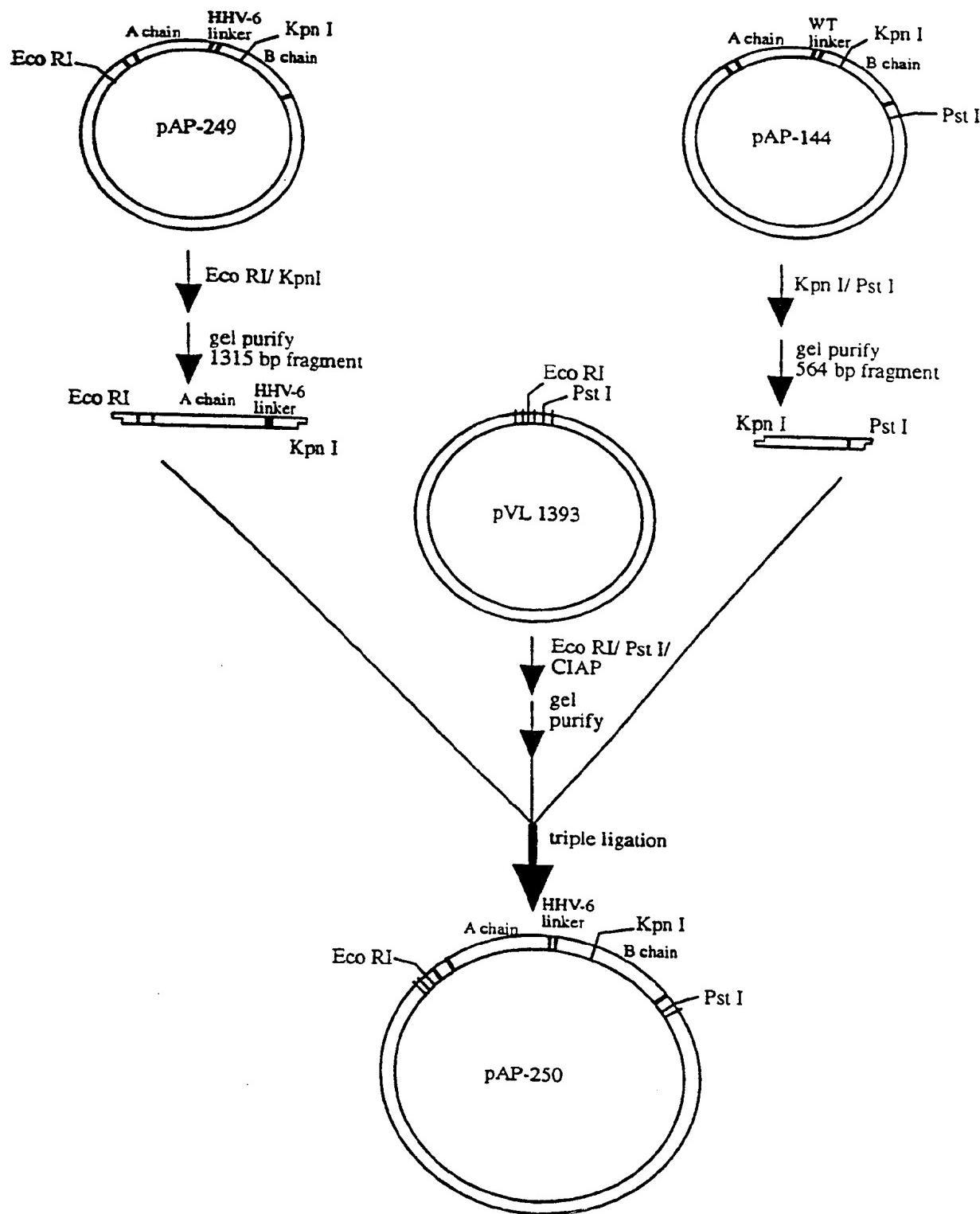
97/254

**FIGURE 20A**

98/254

**FIGURE 20B****WT preorocin linker**

99/254

**FIGURE 20C**

100/254

FIGURE 20D

10	20	30	40	50
1 GAATT	CATGAAACGGGAGGAA	ACTATTGT	ATATGGATGT	TATGCAGT
	CTTAAGTACTTTGCCCTC	TTATGATAACATTATA	ACCTACATACGTCA	
51 GGCAACATGGCTTGT	TTGGATCCACCTCAGGGTGG	GTCTTCACATTAG		
	CCGTTGTACCGAAACAA	ACCTAGGTGGAGTCCC	ACCAGAAAGTGTAA	TC
101 AGGATAACAACATATT	CCCCAACAAATACCA	ATTATAAACTTTACCA	ACA	
	TCCTATTGTTGTATAAGGG	GGTTATGGGTTAATAT	TTGAAATGGTGT	
151 GCGGGTGCCACTGTG	CAAAGCTACACAA	ACTTTATCAGAGCTG	TTCGGG	
	CGCCCCACGGTACACG	TTCGATGTGT	TTGAAATAGTCTCG	ACAAGCGCC
201 TCGTTAACAACTGGAG	CTGATGTGAGACATG	ATATACCAGTGT	GTTGCCAA	
	AGCAAATTGTTGACCT	CGACTACACTCTGT	ACTATATGGT	CACAACGGTT
251 ACAGAGTTGGTTGC	CTATAAACCAACGG	TTTATTTAGTTGA	ACTCTCA	
	TGTCTCAACCAACGG	ATATTGGTGC	CAAATAAAATCA	ACTTGAGAGT
301 AATCATGCAGAGCTT	TCTGTATACATTAGCG	GCTGGATGT	CACCAATGCATA	
	TTAGTACGTCTCGAA	AGACATGT	ATCGCACCTACAGT	GGTTACGTAT
351 TGTGGTCGGCTACCG	TGTGGAAATAGCG	CATATTCTTCAT	CCTGACA	
	ACACCAGCCGATGGC	ACGACCTTATCGCG	TATAAAGAAAGTAGG	ACTGT
401 ATCAGGAAGATGC	AAGCAATCACTCAT	CTTTCACTGATG	TGTTCAAAT	
	TAGTCCTTCTACGT	CTCGTTAGT	GAGTAGAAAAGT	GACTACAAGTTTA
451 CGATATACATTGCCTT	GGGTAATTATGATAGA	CTTGAAACAAC	TTGC	
	GCTATATGTAAGCGGAA	ACCACCAATTAA	ACTATCTGAACT	TGTTGAACG
501 TGGTAATCTGAGAG	AAAATATCGAGTTGG	AAATGGTCCACTA	GGAGGAGG	
	ACCATTAGACTCT	TTTATAGCTAAC	CCCTTACCA	GGGTGATCTCC
551 CTATCTCAGCGCTT	TATTATTAACAGT	ACTGGTGGCACTCAG	CTTCCA	ACT
	GATAGAGTCGCGAA	ATAATAATGT	CATGACCACCG	TGAGTCGAAGGTTGA
601 CTGGCTCGTCTTTA	ATTGCATCCAAATG	ATTTCAGAAGCAG	CAAG	
	GACCGAGCAAGGAA	ATATTAAACG	TAGGTTACTAAAGT	CTTCGTCGTT
651 ATTCCAATATATTG	GAGGGAGAAATGCG	CACGAGAATTAGGT	ACAACCGGA	
	TAAGGTTATATAACT	CCCTCTTACCGGT	GCTCTTAATCC	ATGTTGGCCT
701 GATCTGCACCAGATC	CTAGCGTAATTAC	ACTTGAGAATAGT	TGGGGAGA	
	CTAGACGGTCTAGG	ATCGCATTATGT	GA	ACTCTTATCAACCCCTCT
751 CTTTCCACTGCAATT	CAAGAGTCTAACCA	AGGAGCCTTGCTAG	TCCAAT	
	GAAAGGTGACGTTA	AGTTCTCAGATTGG	TCCCTGGAAACGAT	CAGGTTA
801 TCAACTGCAAAGAC	GTAAATGGTCCA	AAATTCA	GTGACGATGTGAG	TA
	AGTTGACGTTCTGC	ATTACCAAGGTTA	AGTCACACATG	CTACACTCAT
851 TATTAATCCCTATC	ATAGCTCATGGT	GTATAGATGCG	CACCTCCACCA	
	ATAATTAGGGATAGT	CGAGAGTACCA	CACATATCTAC	CGGTGGAGGTGGT
901 TCGTCACAGTTTCTC	GATTTAAATGCATGG	TGCCAATT	TTAATGC	
	AGCAGTGTCAAAGCT	AAATTACGTTAGC	GCACGGTTAAAATTACG	

101/254

**FIGURE 20D (CONT'D)**

951 TGATTTGTATGGATCCTGAGCCCATAGTCGTATCGTAGGTCGAAATG  
 ACTACAAACATAACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
 CAGATACACAACATAACCTACCTCTAAGGTGTTGCCCTTGCCTTAC  
 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTAGATTATGTCAGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAAATGC  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAAAACTGCTGCA  
 CCATGTCAGGCCCTCAGATACACTAGATAACGTTATGACGACGTC  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC  
 TGACTACGGTGGCGACCCTTATACCCCTATTACCTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTAGCAGCGACATCAGGAAACAGTGGTACACAC  
 GTCTAGATCAGATCAAATCGTCGCTGAGTCCCTGTCACCAGGGTGTG  
 1301 TTACAGTGCAACCAACATTATGCCGTTAGTCAGGTTGGCTTCTACT  
 AATGTCACGTTGGTTGTAACAGGCAATCAGTTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
 TTATTATGTTGGAAAACATGTTGGTAACACCCGATATACCAACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
 GAACGTTCGTTATCACCTGTCATACCTATCTCTGACATCGTCACTTT  
 1451 AGGCTGAACAAACAGTGGCTTTATGCAGATGGTCAATACGTCCTCAG  
 TCCGACTTGTGTCACCCGAGAAATACGTCACCAAGTTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGATTCTAATATACGGAAACAGT  
 GTTTGGCTCTATTAACGGAATGTTCACTAAGATTATGCCCTTGTCA  
 1551 TGTTAACGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
 ACAATTCTAGGAGAGAACACCGGACGTAGGAGACCGGTTGCTACCTACA  
 1601 TCAAGAACATGGAACCATTAAATTGTTAGTGATAGTGGATTGGTGTAGAT  
 AGTTCTTACTACCTGGTAAATTAAACATATCACCTAACACAATCTA  
 1651 GTGAGGCAGTCGGATCCGAGCCTTAAACAAATCATTCTTACCCCTCTCCA  
 CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
 1701 TGGTGACCCAAACCAAATATGGTACCAATTATTTGATAGACAGATTACT  
 ACCACTGGGTTGGTTATACCAATGGTAAATAAAACTATCTGTCTAATGA  
 1751 CTCTTGCACTGTGTTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAAA  
 GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATTTT  
 1801 GGACATTGTAATTGTAACGTGAAAGGACAGCAAGTTATATCGAATTCC  
 CCTGTAACATTAAACATTGACTTCTGTCGTTCAATATAGCTTAAGG  
 1851 TGCAG  
 ACGTC

102/254

**FIGURE 21**

Ricin linker (wild type) :

A chain- S L L I R P V V P N F N -B chain

pAP-213/pAP-214 linker (Cathepsin B) :

A chain- S L L K S R M V P N F N -B chain

pAP-215/pAP-216 linker (MMP-3) :

A chain- R P K P Q Q F F G L M N -B chain

pAP-217/pAP-218 linker (MMP-7) :

A chain- S L R P L A L W R S F N -B chain

pAP-219/pAP-220 linker (MMP-9) :

A chain- S P Q G I A G Q R N F N -B chain

pAP-221/pAP-222 linker (THERMOLYSIN-LIKE MMP) :

A chain- D V D E R D V R G F A S F L -B chain

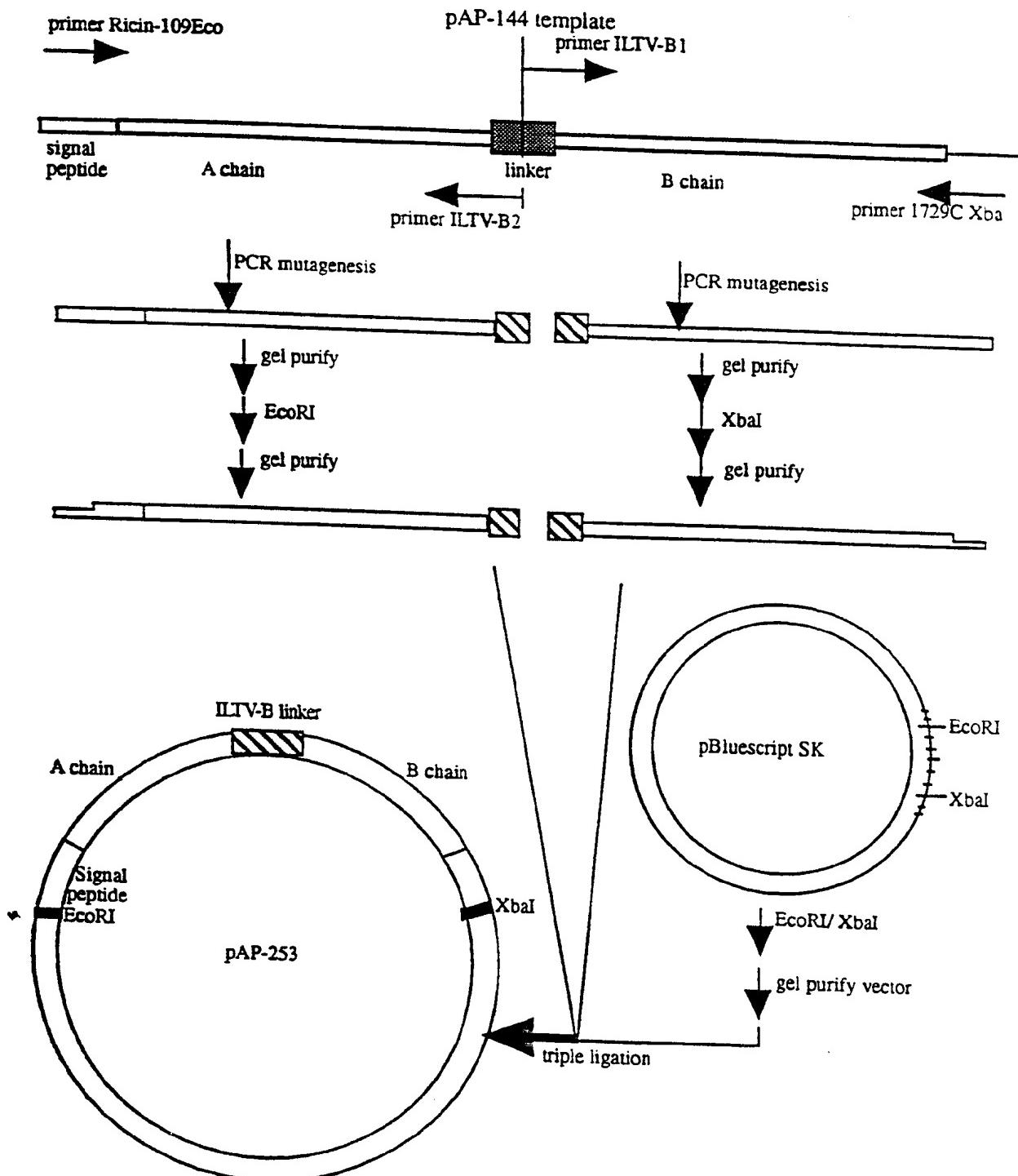
pAP-241/pAP-242 linker (EBV-A) :

A chain- S K L V Q A S A S G V N -B chain

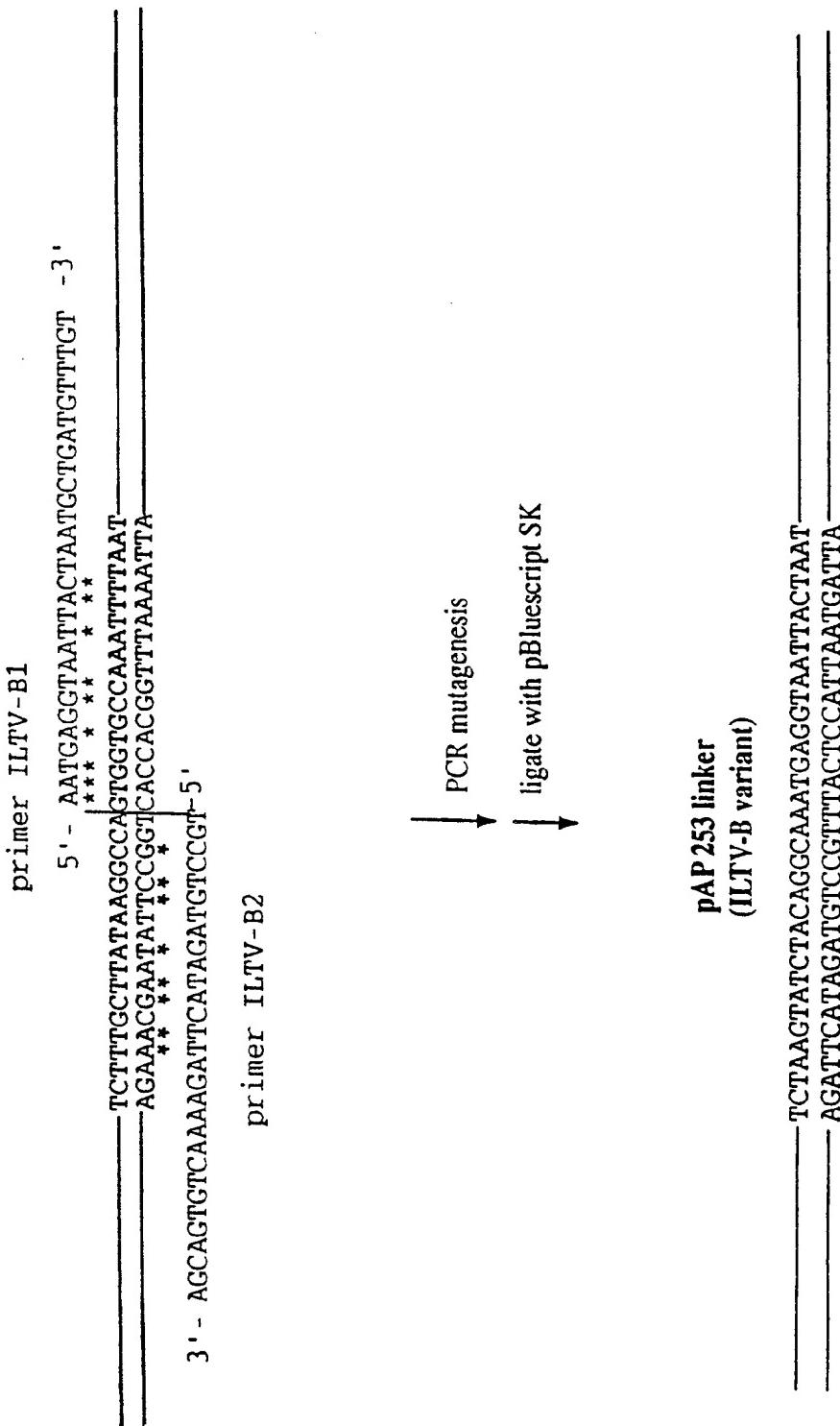
pAP-243/pAP-244 linker (EBV-B) :

A chain- S S Y L K A S D A P D N -B chain

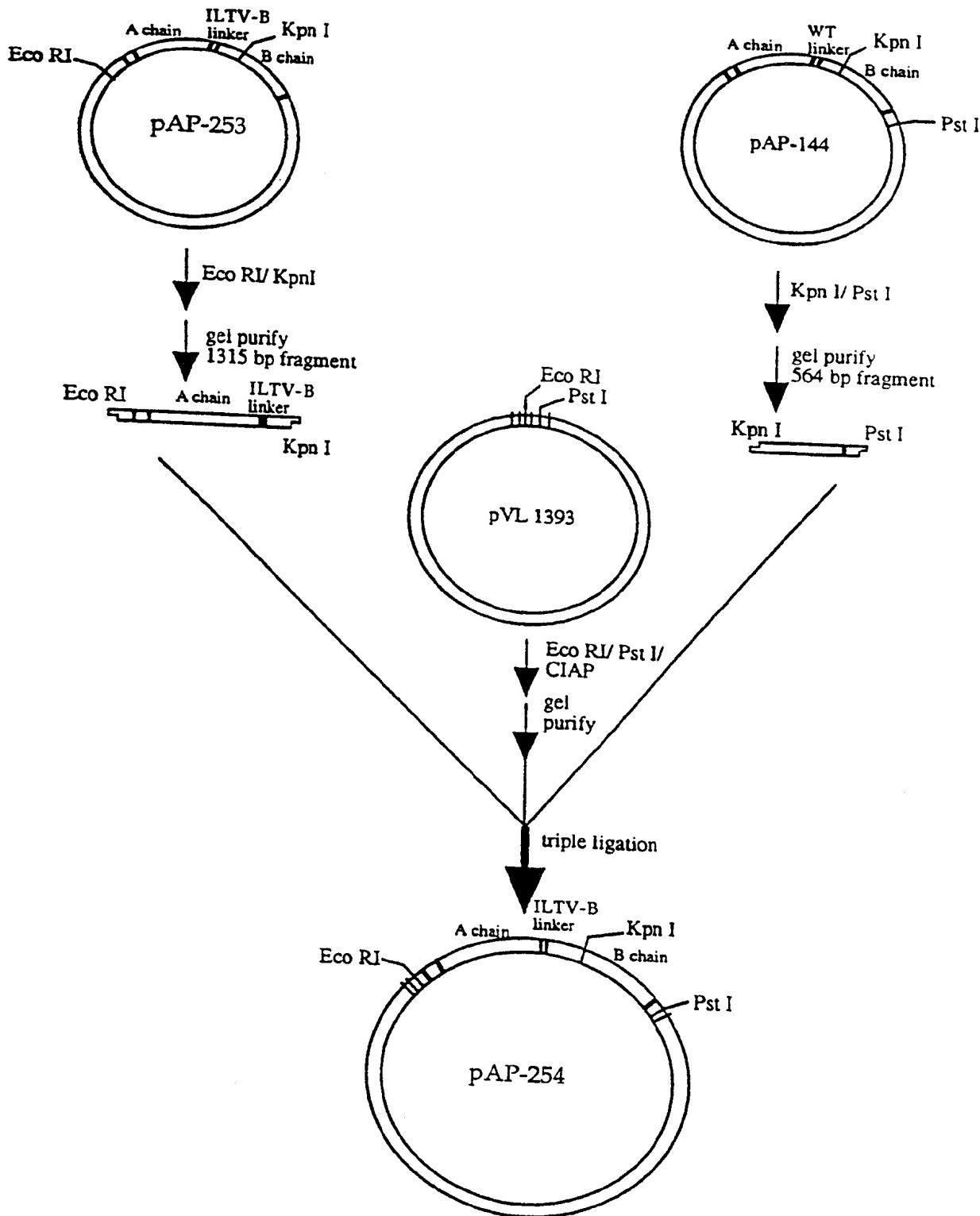
103/254

**FIGURE 22A**

104/254

**FIGURE 22B****WT prepricin linker**

105/254

FIGURE 22C

106/254

FIGURE 22D

1	10	20	30	40	50
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GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGCCCTCCTTTATGATAACATTACACATACGTCA

51 GGCAACATGGCTTGGATCCACCTCAGGGTGGCTTCACATTAG
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC

101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTACCA
TCCTATTGTTGTATAAGGGGTTGTTATGGGTTAATATTGAAATGGTGT

151 GCGGGTGCCACTGTCAAAGCTACACAAACTTTATCAGAGCTGTTCGCG
CGCCCAACGGTGACACGTTCGATGTGTTGAAATAGTCTCGACAAGCGCC

201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATAACCAGTGGTCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACCTCTCA
TGTCTCAACCAAACGGATATTGGTGCCAAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCACCTACAGTGGTACGTAT

351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTCTTCATCCTGACA
ACACCAGCGATGGCACGACCTTATCGGTATAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTCACTGATGTTAAAAT
TAGTCCTTCTACGTCCTCGTTAGTGAGTAGAAAGTGAACATCAAGTTTA

451 CGATATACTCGCTTGGTGGTAATTATGATAGACTTGAACAACTTGC
GCTATATGTAAGCGGAAACCAACCATTAATACTATCTGAACCTGTTGAACG

501 TGGTAATCTGAGAGAAAATATCGAGTGGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTTTATAGCTCAACCCCTTACAGGTGATCTCCCTCC

551 CTATCTCAGCGCTTTATTACAGTACTGGTGGCACTCAGCTTCAACT
GATAGAGTCGCAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTTCTTATAATTGATCCAAATGATTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAACGTAGGTTACTAAAGTCTCGTGTTC

651 ATTCCAATATATTGAGGGAGAAATGCCACGAGAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTTTACGCGTGTCTTAATCCATGTTGGCCT

701 GATCTGCACCAAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA
CTAGACGTGGCTAGGATCGCATTAAATGTGAACCTTATCAACCCCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGTAGTCAAAT
GAAAGGTGACGTTAAGTCTCAGATTGGTCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTCCAATTCACTGAGTGTACGATGTGAGTA
AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCCACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTCTAAGTATCTACAGGCAAATGAGGTAAATTACTAATG
AGCAGTGTCAAAGATTAGATGTCGTTACTCCATTAAATGATTACG

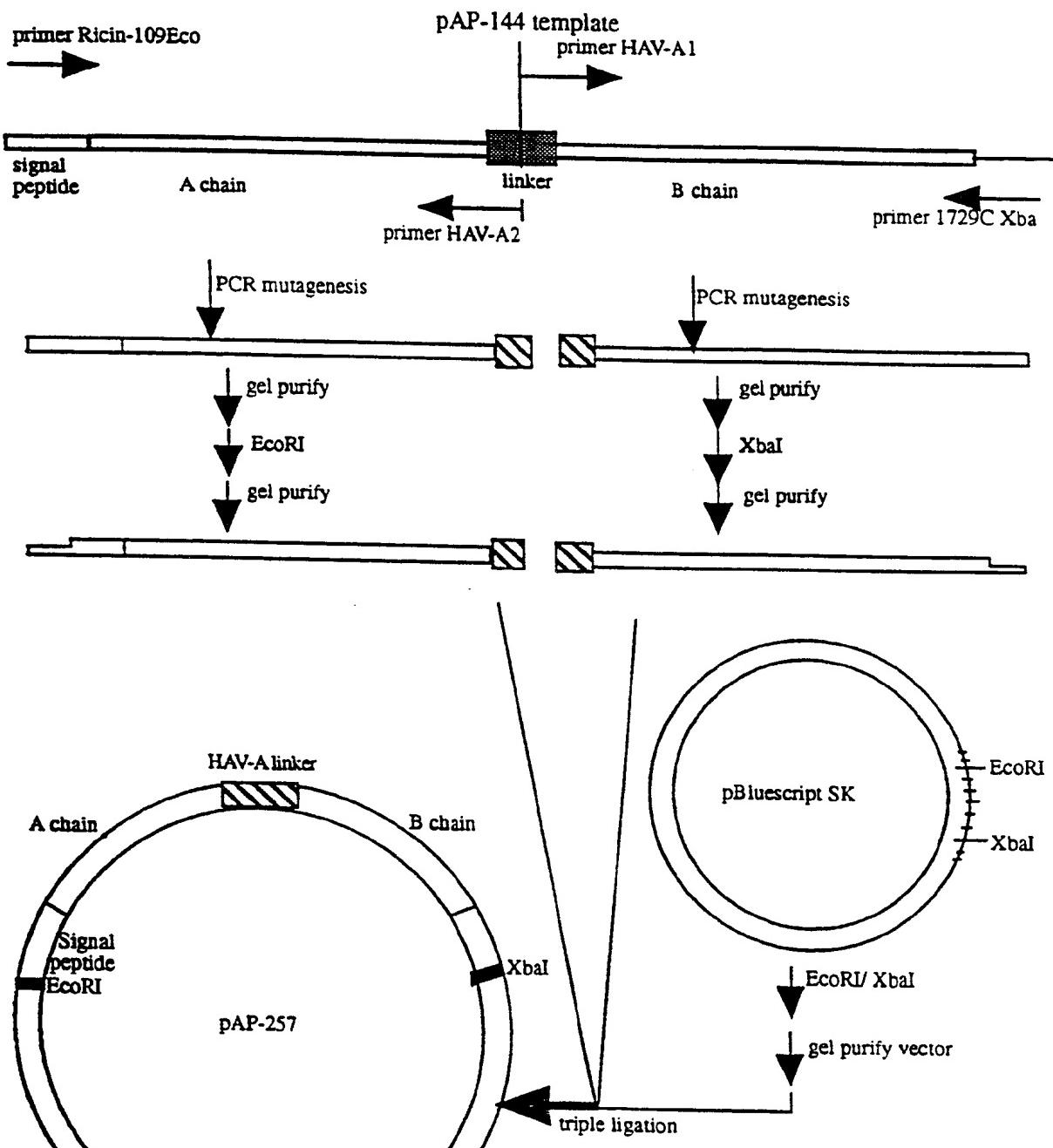
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107/254

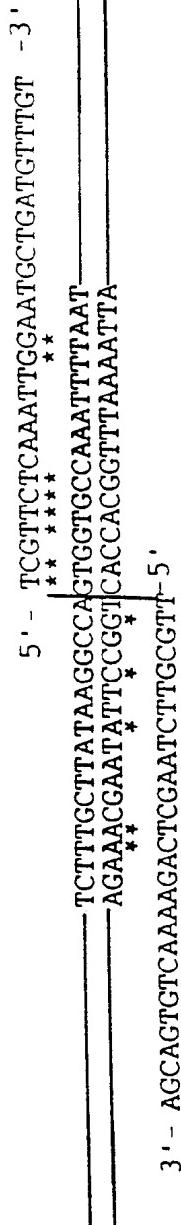
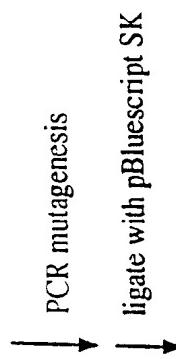
**FIGURE 22D (CONT'D)**

951 TGATTTGTATGGATCCTGAGCCCATAGTCGTATCGTAGGTCGAAATG  
     ACTACAAACATAACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
     CAGATACACAACATAACATCCCTACCTCTAAGGTGTTGCCCTTGCCTTAT  
 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
     GTCAACACCAGGTACGTTACGATTATGCTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGAAAGTGTAACTACTTACG  
     CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGAAATACTGCTGCA  
     CCATGTCAGGCCCTCAGATACTACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC  
     TGACTACGGTGGCGACCGTTATACCTTACCTTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGAAACAGTGGTACCAACAC  
     GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTACCATGGTGTG  
 1301 TTACAGTGCACCAACACATTATGCCGTTAGTCAGGTTGGCTTCCTACT  
     AATGTCACGTTGGTTGTAATACGGAATCAGTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
     TTATTATGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
     GAACGTTCGTTATCACCTGTCATACTATCCTGACATCGTCACCTT  
 1451 AGGCTGAACAACAGTGGCTCTTATGCAAGATGGTTCAATACGTCTCAG  
     TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGATTCTAAATATACGGAAACAGT  
     GTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTGTCA  
 1551 TGTTAAGATCCTCTCTGTTGGCCCTGCATCCTCTGCCAACGATGGATGT  
     ACAATTCTAGGAGAGAACACCCGGACGTAGGAGACGGTTGCTACCTACA  
 1601 TCAAGAATGATGGAACCATTAAATTGTTAGTGGATTGGTGTAGAT  
     AGTTCTTACTACCTTGGTAAATTAACATATCACCTAACACAATCTA  
 1651 GTGAGGCAGTCGGATCCGAGCCTAAACAAATCATTCTTACCCCTCTCCA  
     CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
 1701 TGGTGAACCAAACAAATATGGTACCAATTGTTAGTGGATAGACAGATTACT  
     ACCACTGGGTTGGTTATACCAATGGAATAAAACTATCTGTCTAATGA  
 1751 CTCTTGCAGTGTGTTGCTGCCATGAAAATAGATGGCTTAAATAAAAAA  
     GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATTTT  
 1801 GGACATTGTAATTTGTAACGTGAAAGGACAGCAAGTTATATCGAATTCC  
     CCTGTAACATTAAAACATTGACTTCCCTGTCGTTCAATATAGCTTAAGG  
 1851 TGCAG  
     ACGTC

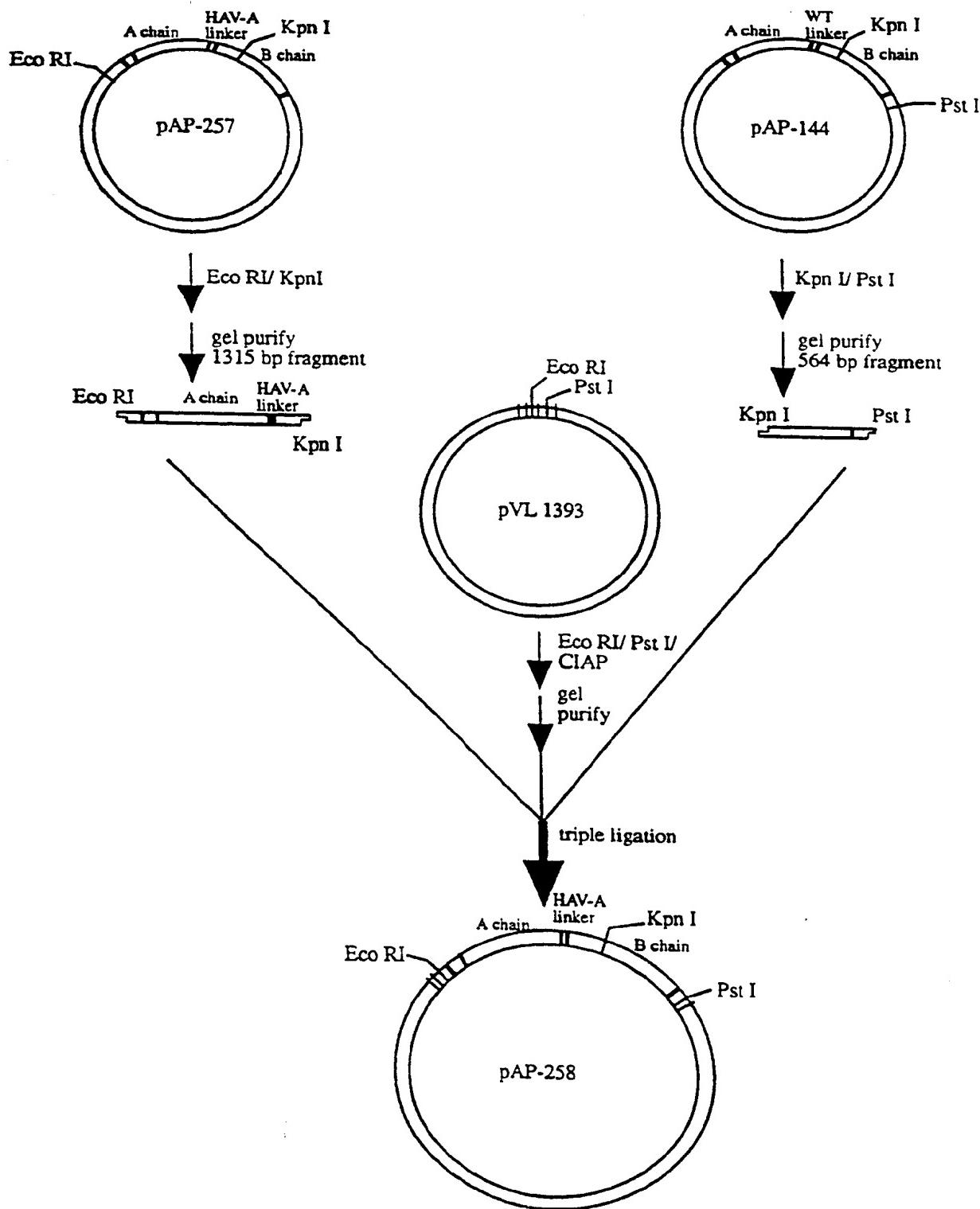
108/254

FIGURE 23A

109 / 254

**FIGURE 23B****WT preprotein linker****primer HAV-A1****primer HAV-A2****pAP 257 linker  
(HAV-A variant)**

110/254

**FIGURE 23C**

111/254

FIGURE 23D

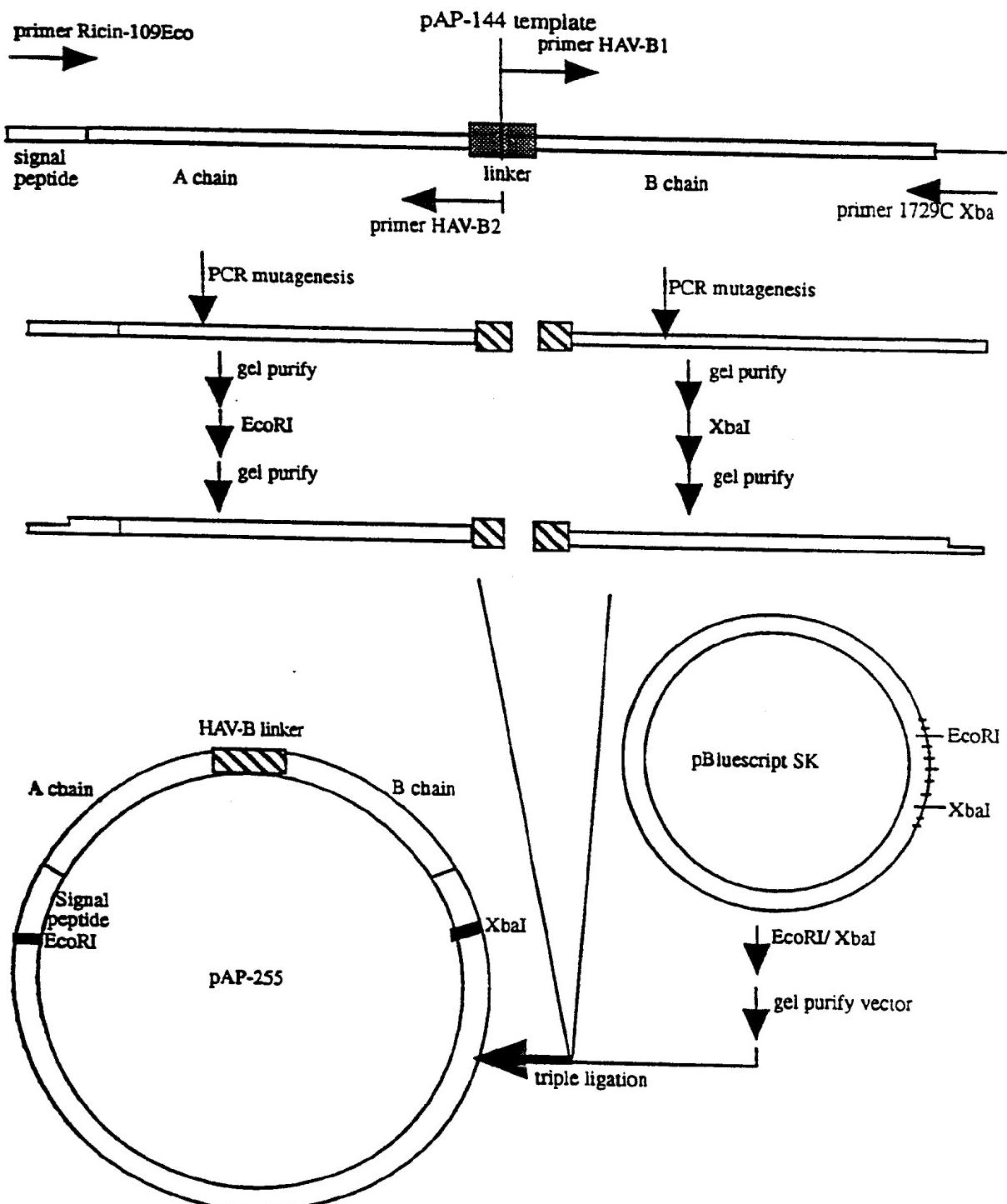
10	20	30	40	50
1 GAATT	CATGAAACGGGGAGGAAATACTATTGTAATATGGATGTATGCAGT			
	CTTAAGTACTTTGCCCTCCTTATGATAACATTACACATACAGTC			
51 GGCAACATGGCTTGTGATCCACCTCAGGTGGTCTTCACATTAG				
	CCGTTGTACCGAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGAATC			
101 AGGATAACAACATATTCCCCAACAAATACCCAATTATAAACTTACCA				
	TCCTATTGTTGATAAGGGGTTGTTATGGGTTAATATTGAAATGGTGT			
151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTTATCAGAGCTGTTGCCG				
	CGCCCACGGTGACACGTTGATGTGTTGAAATAGTCTCGACAAGCGCC			
201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCACTGTGTTGCCA				
	AGCAAATTGTTGACCTCGACTACACTCTGACTATATGGTCACAACGGTT			
251 ACAGAGTTGGTTGCCATAAACCAACGGTTATTTAGTTAGTGAACCTCA				
	TGTCTCAACCAACGGATATTGGTTGCCAAATAAAATCAACTTGAGAGT			
301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA				
	TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT			
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTCTTCATCCTGACA				
	ACACCAGCCGATGGCACGACCTTATCGGTATAAAAGAAAGTAGGACTGT			
401 ATCAGGAAGATCCAGAAGCAATCACTCATTTTCACTGATGTTCAAAAT				
	TAGTCCTTCTACGTCTCGTTAGTAGAAAGTGAACACTACAAGTTTA			
451 CGATATACATTGCCCTTGGTGGTAATTATGATAGACTTGAACAACTTGC				
	GCTATATGTAAGCGAACCCACCATTAATACTATCTGAACCTGTTGAACG			
501 TGGTAATCTGAGAGAAAATATGAGTTGGAAATGGTCCACTAGAGGAGG				
	ACCATTAGACTCTTTATAGCTCAACCCCTTACAGGTGATCTCCCTCC			
551 CTATCTAGCGCTTATTATTACAGTACTGGTGGCACTCAGCTTCAACT				
	GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTG			
601 CTGGCTCGTCCATTATAATTGCATCCAAATGATTCAGAACAGCAGCAAG				
	GACCGAGCAAGGAAATATTAACGTAGGTTACTAAAGTCTCGTCGTT			
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAAATTAGGTACAACCGGA				
	TAAGGTTATATAACTCCCTTTACCGTGCTCTTAATCCATGTTGGCCT			
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAAATAGTTGGGGAGA				
	CTAGACGTGGCTAGGATCGCATTAAATGTGAACCTTATCAACCCCCCTCT			
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCAAAT				
	GAAAGGTGACGTTAAGTCTCAGATTGGTCCCTCGAACGATCAGGTTA			
801 TCAACTGCAAAGACGTAATGGTCCAAATTCACTGTGAGTACGATGTGAGTA				
	AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT			
851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA				
	ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACCGTGAGGTGGT			
901 TCGTCACAGTTCTGAGCTTAGAACGCAATCGTCTCAAATTGGAATGC				
	AGCAGTGTAAAAGACTCGAATCTCGTTAGCAAGAGTTAACCTACG			

112/254

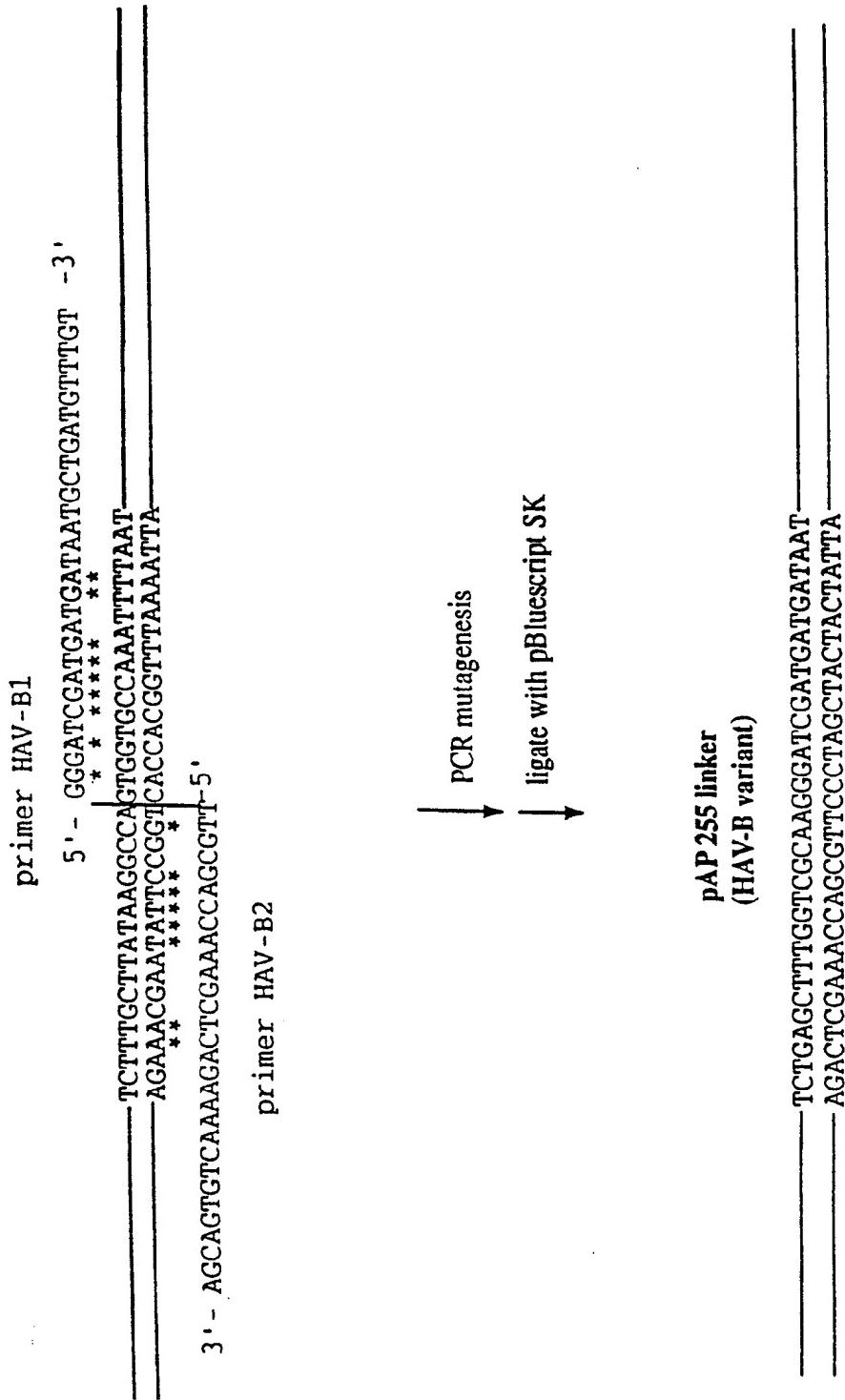
FIGURE 23D (CONT'D)

951 TGATGTTTGTATGGATCCTGAGCCCATACTGCGTATCGTAGGTCGAAATG  
 ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
 CAGATACACAACATACTACCTTCTAAGGTGTTGCCTTGCGTTAT  
 1051 CAGTTGTGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTAGATTATGTCAGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA  
 CCATGTCAGGCCCTCAGATACTACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGATAATGGAACCATCATAAATCC  
 TGACTACGGTGGCGACCGTTATACCTTACCTTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
 GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTACCATGGTGTG  
 1301 TTACAGTCAAACCAACATTATGCCGTTAGTCAAGGTTGGCTTCTACT  
 AATGTCACGTTGGTTGTAACGGCAATCAGTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
 TTATTATGTTGGAAAACAATGTTGTAACAAACCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAAGTATGGATAGAGGACTGTAGCAGTAAA  
 GAACGTTCGTTATCACCTGTTACACCTATCTCCTGACATCGTCACTTT  
 1451 AGGCTGAACAACAGTGGCTCTTATGCAGATGGTTCAATACTGCTCAG  
 TCCGACTTGTGTCACCCGAGAAATACGTCACCAAGTTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGATTCTAATATAACGGAAACAGT  
 GTTTGGCTCTATTAAACGGAATGTCACTAAGATTATATGCCCTTGTCA  
 1551 TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
 ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA  
 1601 TCAAGAATGATGGAACCATTAAATTGATAGTGGATTGGTGTAGAT  
 AGTTCTTACTACCTTGGTAAATTAAACATATCACCTAACCAATCTA  
 1651 GTGAGGCATGGATCCGAGCCTAAACAAATCATTCTTACCCCTCTCCA  
 CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
 1701 TGGTGACCAAACCAAATATGGTTACCAATTATTGATAGACAGATTACT  
 ACCACTGGGTTGGTTACCAATGGTAATAAAACTATCTGTCTAATGA  
 1751 CTCTTGCAGTGTGTGTCCTGCCATGAAAATAGATGGCTAAATAAAAAA  
 GAGAACGTCACACACAGGACGGTACTTTATCTACCGAATTATTTT  
 1801 GGACATTGTAATTGTAACGAAAGGACAGCAAGTTATATCGAATTCC  
 CCTGTAACATTAAAACATTGACTTTCTGTCGTTCAATATAGCTTAAGG  
 1851 TGCAG  
 ACGTC

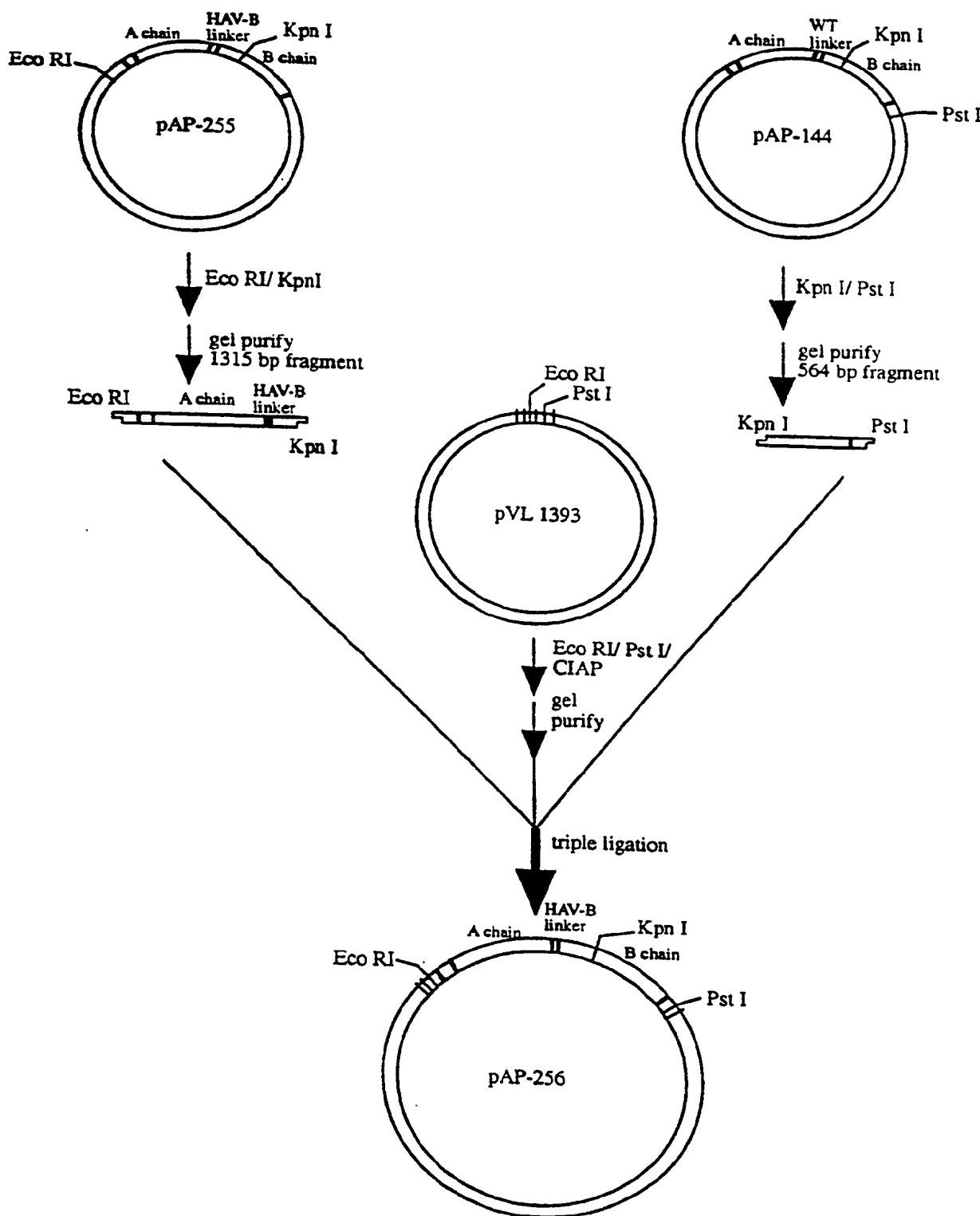
113/254

**FIGURE 24A**

114/254

**FIGURE 24B****WT preprorin linker**

115/254

**FIGURE 24C**

116/254

**FIGURE 24D**

1	10	20	30	40	50
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GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
CTTAAGTACTTTGCCCTCCTTATGATAACATTATACTACATACGTCA

51 GGCAACATGGCTTGTGATCCACCTCAGGGTGGCTTCACATTAG
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC

101 AGGATAACAACATATTCCCCAAACARTACCCAATTATAAACTTTACCACA
TCCTATTGTTGATAAGGGTTTGTATGGTTAATATTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTTATCAGAGCTGTTGCCGG
CGCCCACGGTGACACGTTCGATGTGTTGAAATAGTCTGACAAGCGCC

201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATAACCAGTGTGCAA
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACCTCTCA
TGTCTAACCAACGGATATTGGTTGCCAAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGCCTGAAATAGCGCATATTCTTCATCCTGACA
ACACCAGCCGATGGCACGACTTTATCGCGTATAAGAAAGTAGGACTGT

401 ATCAGGAAGATGAGAAGCAATCACTCATCTTCACTGATGTTCAAAT
TAGTCCTTCTACGTCTCGTTAGTGAAGTAGAAAGTGAACACTAAAGTTTA

451 CGATATACATTGCCCTTGGTGGTAATTATGATAGACTTGAAACAACCTGC
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACCTGTTGAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG
ACCATTAGACTCTTTATAGCTAACCCCTTACCGGTGATCTCCTCC

551 CTATCTCAGCGCTTTATTACAGTACTGGTGGCACTCAGCTTCAA
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTGTA

601 CTGGCTCGTTCTTATAATTGCAATCCAAATGATTCAGAAGCAGCAAG
GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTGTTC

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAAATTAGGTACAACCGGA
TAAGGTTATATAACTCCCTCTTACCGGTGCTTTAATCCATGTTGGCCT

701 GATCTGCAACAGATCCTAGCGTAATTACACTTGAGAAATAGTTGGGGAGA
CTAGACGTGGTCTAGGATCGCATTAAATGTAACCTTATCAACCCCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCAAAT
GAAAGGTGACGTTAAGTCTCAGATTGGTCCCTCGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTCCAAATTCACTGAGTGTACGATGTGAGTA
AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACCGGTGGAGGTGGT

901 TCGTCACAGTTTCTGAGCTTGGTGCAGGGATCGATGATGATAATGC
AGCAGTGTCAAAGACTCGAAACCAGCGTCCCTAGCTACTACTATTACG

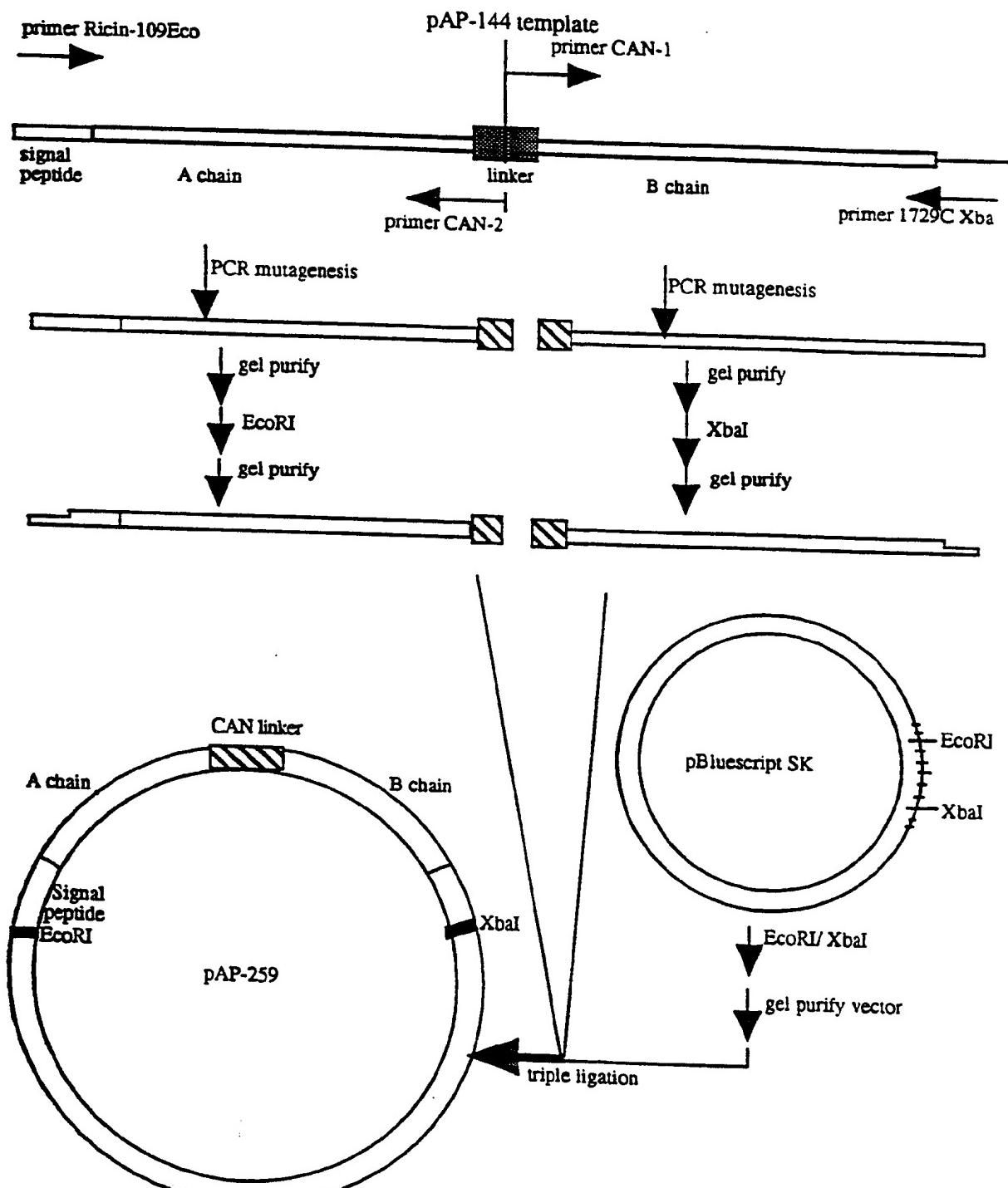
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117/254

**FIGURE 24D (CONT'D)**

951 TGATGTTGTATGGATCCTGAGCCCATAGTCGTATCGTAGGTCGAAATG  
 ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGAAACGCAATA  
 CAGATACACAACATACTACATCCCTACCTCTAAGGTGTTGCCTTGCGTTAT  
 1051 CAGTTGTGCCATGCAAGCTAATAACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTACGATTATGCTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAAAACTGCTGCA  
 CCATGTCAGGCCCTCAGATACACTACTAGATAACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCACATCATAAATCC  
 TGACTACGGTGGCGACCGTTATACCTTACCTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAAACAGTGGTACCAACAC  
 GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTTGTCAACCAGGTGTG  
 1301 TTACAGTGCAAAACCAACATTATGCCGTTAGTCAGGTTGGCTTCTACT  
 AATGTCACGTTGGTTGTAATAACGGAATCAGTCCAAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATAATGGTCTGTG  
 TTATTATGTTGGAAAACAATGTTGGTAACAACCCGATATAACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
 GAACGTTGCTTATCACCTGTTACACCTATCTCCTGACATCGTCACTT  
 1451 AGGCTGAACAAACAGTGGCTCTTATGCAGATGGTCAATACTCCTCAG  
 TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
 1501 CAAACCGAGATAATTGCCCTACAAGTGAATTCTAATATAACGGAAACAGT  
 GTTTGGCTCTATTAAACGGAATGTTACTAAGATTATATGCCCTTGTCA  
 1551 TGTTAAGATCCTCTTGTCGGCCCTGCATCCTCTGGCCAACGATGGATGT  
 ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA  
 1601 TCAAGAATGATGGAACCATTAAATTGTTAGTGGATGGTGTAGAT  
 AGTTCTTACTACCTGGTAAATTAACATATCACCTAACCAACAACTCA  
 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTACCCCTCCA  
 CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
 1701 TGGTGAACCAACAAATATGGTTACCAATTATTTGATAGACAGATTACT  
 ACCACTGGGTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA  
 1751 CTCTTGCAGTGTGTTGTCCTGCCATGAAAATAGATGGCTAAATAAAAAA  
 GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATTTT  
 1801 GGACATTGTAATTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC  
 CCTGTAACATTAAAACATTGACTTCTGTCGTTCAATATAGCTTAAGG  
 1851 TGCAG  
 ACGTC

118/254

**FIGURE 25A**

119 / 254

## **FIGURE 25B**

### WT preprorocin linker

primer CAN-1

5'	-	TTCAAGGCTAAATTATGGCTGAT	-3'
	*	*	*
		TCTTTGCTTATAAGGCCAATTTAAAT	
		AGAACGAAATTCCGGTACACACGGTTAAAATTAA	
		****#*#*	
		*****	

primer CAN-2

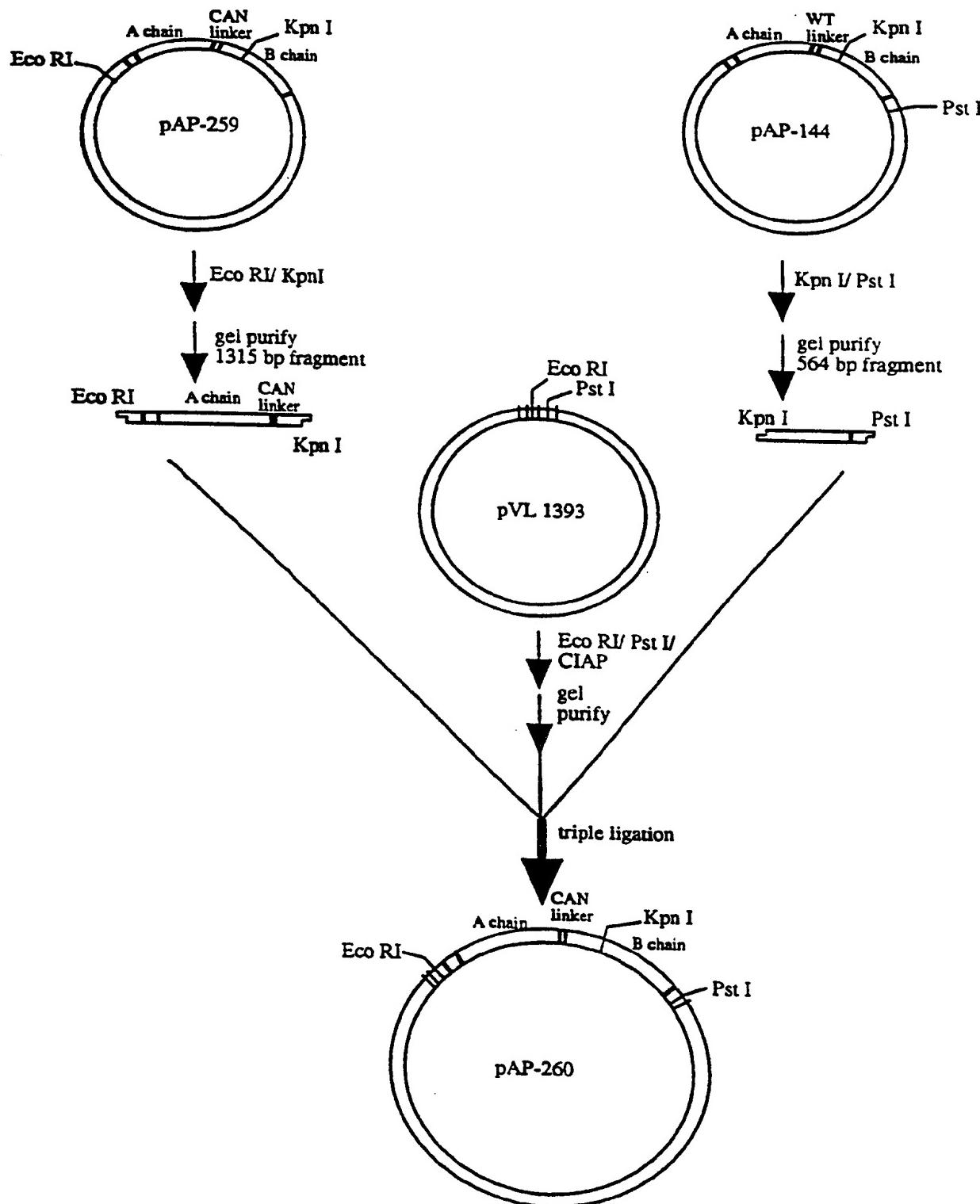
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graph TD
    A[PCR mutagenesis] --> B[ligate with pBluescript SK]
  
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pAP 259 linker  
(CAN variant)

TCTAAGGCCGCAAGTTCTTACGGCTAAATTAAAT  
AGATTGGACGGTTCAAGAACGTCCGGATTAAATTA

120/254

**FIGURE 25C**

121/254

**FIGURE 25D**

1	10	20	30	40	50
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GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT  
 CTTAAGTACTTGGCCCTCCTTATGATAACATTATACCTACATACGTCA  
 GGCAACATGGCTTGTTGGATCCACCTCAGGGTGGCTTTCACATTAG  
 CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC  
 AGGATAACAACATATTCCCCAACAAACCCAATTATAAACTTACCAAC  
 TCCTATTGTTGATAAGGGTTGTTATGGTTAATATTGAAATGGTGT  
 GCGGGTGCCTACTGTGCAAAGCTACACAAACTTATCAGAGCTGTCGCC  
 CGCCACGGTGACACGTTGATGTGTTGAAATAGTCTGACAAGCGCC  
 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCAAGTGTGCCAA  
 AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT  
 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACCTCTCA  
 TGTCTCAACCAAACGGATATTGGTTGCCAACATAAAACTGAGAGT  
 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA  
 TTAGTACGTCTCGAAAGACAATGTAATCGCACCTACAGTGGTTACGTAT  
 TGTGGTCGGTACCGTCTGGAAATAGCGCATATTCCTCATCCTGACA  
 ACACCAGCCGATGGCACGACCTTATCGGTATAAGAAAGTAGGACTGT  
 ATCAGGAAGATGCAGAACATCACTCATCTTCACTGATGTTCAAAAT  
 TAGTCCTCTACGTCTCGTTAGTAGAAAAGTAGACTACAAGTTTA  
 CGATATACATTGCCCTTGGTGTAAATTATGATAGACTTGAACAACTTGC  
 GCTATATGTAAGCGAACCCACCATTAATACTATCTGAACCTGTTGAACG  
 TGGTAATCTGAGAGAAAATCGAGTGGGAAATGGTCCACTAGAGGAGG  
 ACCATTAGACTCTCTTATAGCTCACCCCTTACAGGTGATCTCCTCC  
 CTATCTCAGCGCTTATTACAGTACTGGTGGCACTCAGCTTCCA  
 GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA  
 CTGGCTCGTCTTTATAATTGCACTCCAAATGATTTAGAAGCAGCAAG  
 GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTCGTT  
 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA  
 TAAGGTTATATAACTCCCTTTACCGTGCTTAATCCATGTTGGCCT  
 GATCTGCACCAGATCTACGTAATTACACTTGAGAATAGTTGGGGAGA  
 CTAGACGTGGTCTAGGATCGCATTATGTGAACCTTATCAACCCCTCT  
 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGGCCTTGCTAGTCCAAT  
 GAAAGGTGACGTTAAGTCTCAGATTGGTCTCGGAAACGATCAGGTTA  
 TCAACTGCAAAGACGTAATGGTCCAAATTCACTGAGTGTACGATGTGAGTA  
 AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT  
 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA  
 ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACCGTGAGGTTG  
 TCGTCACAGTTCTAAGCCTGCAAAGTTCTCAGGCTAAATTAAATGC  
 AGCAGTGTCAAAGATTGGACGTTCAAGAAGTCCGATTAAAATTACG

122/254

**FIGURE 25D (CONT'D)**

951 TGATGTTGTATGGATCCTGAGCCCATACTGCCTAGTCAGGTCGAAATG  
 ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
 CAGATACACAACATACAATCCCTACCTCTAAGGTGTTGCCTTGCGTTAT  
 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTAGATTATGCTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAAACTGCTGCA  
 CCATGTCAGGCCCTCAGATAACACTAGATAACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCGCTGGCAAATATGGGATAATGGAACCATCATAATCC  
 TGACTACGGTGGCGACCCTTATACCTTACCTTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTAGCAGCGACATCAGGAAACAGTGGTACCAACAC  
 GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTCACCATGGTGTG  
 1301 TTACAGTCAAACCAACATTATGCCCTAGTCAGGTTGGCTTCAACT  
 AATGTCACGTTGGTTGAAATACGGCAATCAGTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
 TTATTATGTTGGAAAACAATGTTGTAACAAACCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGGACTGTAGCAGTGAAA  
 GAACGTTCGTTATCACCTGTTACCTATCTCCTGACATCGTCACCTT  
 1451 AGGCTGAACAAACAGTGGCTCTTATGCAGATGGTCAATACGTCCAG  
 TCCGACTTGTGTCACCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGATTCTAATATACGGAAACAGT  
 GTTTGGCTCTATTAAACGGATGTTCACTAAGATTATATGCCCTTGTC  
 1551 TGTTAAGATCCTCTTGTTGGCCCTGCATCCTCTGGCAAACGATGGATGT  
 ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA  
 1601 TCAAGAATGATGGAACCATTTAAATTGTTAGTGGATTGGTGTAGAT  
 AGTTCTTACTACCTTGGTAAATTAACATATCACCTAACCAACAACTCTA  
 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTACCCCTCCA  
 CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
 1701 TGGTGACCAAACCAAATATGGTTACCAATTGTTAGTGGATAGACAGATTACT  
 ACCACTGGGTTGGTTATACCAATGTAATAAAACTATCTGTCTAATGA  
 1751 CTCTTGCAGTGTGTCTGCCATGAAAATAGATGGCTTAAATAAAAAA  
 GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATTTT  
 1801 GGACATTGTAATTTGTAACGTGAAAGGACAGCAAGTTATATCGAATTCC  
 CCTGTAACATTAAAACATTGACTTTCTGTCGTTCAATATAGCTTAAGG  
 1851 TGCAG  
 ACGTC

123/254

**FIGURE 26**

Ricin linker (wild type) :

A chain- S L L I R P V V P N F N -B chain

pAP-223/224 linker (MAL-A) :

A chain- Q V V Q L Q N Y D E E D -B chain

pAP-225/226 linker (MAL-B) :

A chain- L P I F G E S E D N D E -B chain

pAP-227/228 linker (MAL-C) :

A chain- Q V V T G E A I S V T M -B chain

pAP-229/230 linker (MAL-D) :

A chain- A L E R T F L S F P T N -B chain

pAP-231/pAP-232 linker (MAL-E) :

A chain- K F Q D M L N I S Q H Q -B chain

124/254

**FIGURE 27**

Ricin linker (wild type):

A chain- S L L I R P V V P N F N -B chain

pAP-245/246 linker (CMV-A):

A chain- S G V V N A S C R L A N -B chain

pAP-247/248 linker (CMV-B):

A chain- S S Y V K A S V S P E N -B chain

pAP-233/234 linker (HERPES SIMPLEX-1 A):

A chain- S A L V N A S S A H V N -B chain

pAP-235/236 linker (HERPES SIMPLEX-1 B):

A chain- S T Y L Q A S E K F K N -B chain

pAP-249/250 linker (HUMAN HERPES VIRUS-6):

A chain- S S I L N A S V P N F N -B chain

pAP-237/pAP-238 linker (VZV-A):

A chain- S Q D V N A V E A S S N -B chain

pAP-239/pAP-240 linker (VZV-B):

A chain- S V Y L Q A S T G Y G N -B chain

pAP-253/pAP-254 linker (ILV):

A chain- S K Y L Q A N E V I T N -B chain

pAP-255/pAP-256 linker (HAV-A):

A chain- S E L R T Q S F S N W N -B chain

pAP-257/pAP-258 linker (HAV-B):

A chain- S E L W S Q G I D D D N -B chain

125/254

**FIGURE 28**

Ricin linker (wild type) :

A chain- S L L I R P V V P N F N -B chain

pAP-259/260 linker (CAP-A) :

A chain- S K P A K F F R L N F N -B chain

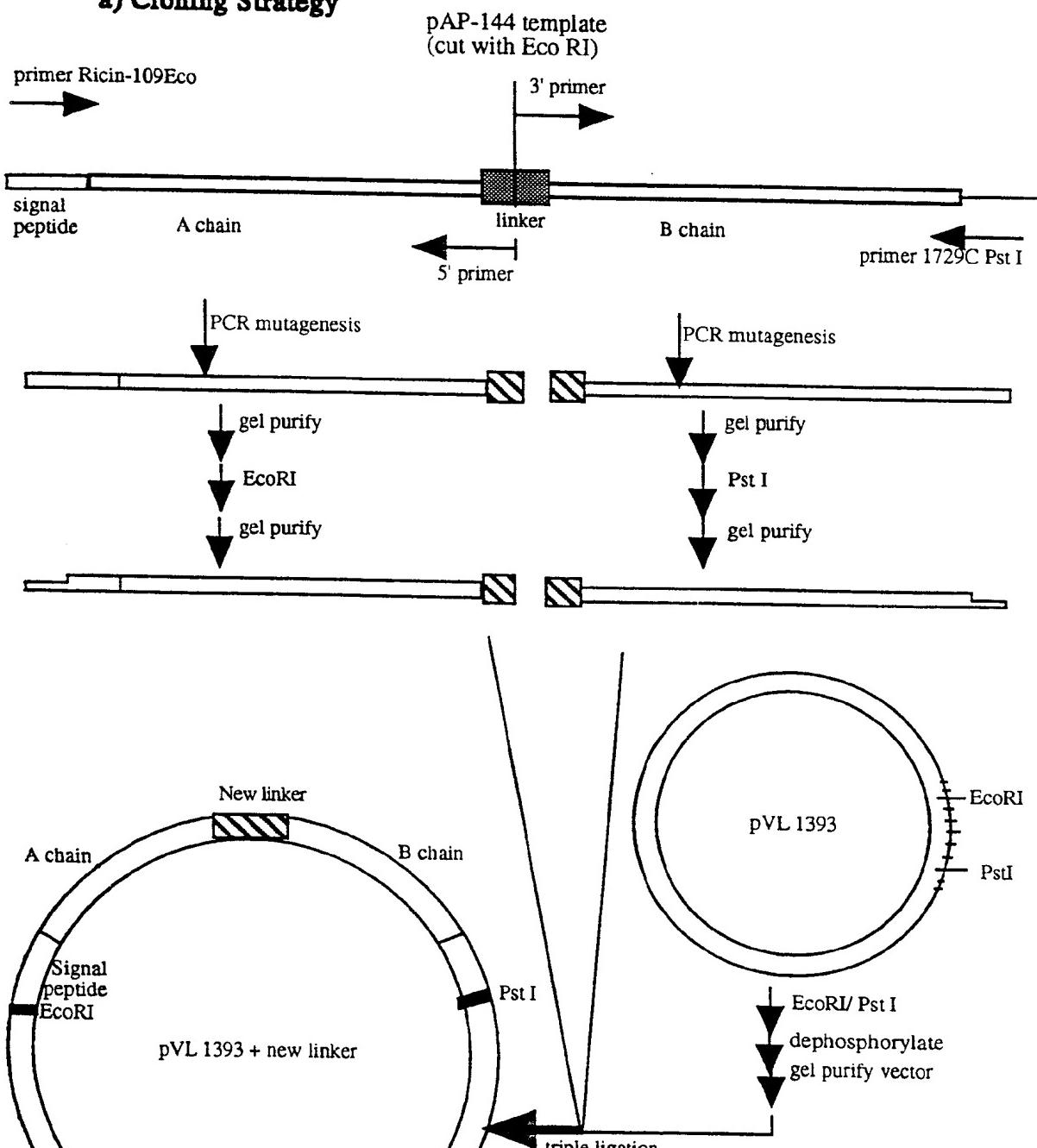
pAP-261/262 linker (CAP-B) :

A chain- S K P I E F F R L N F N -B chain

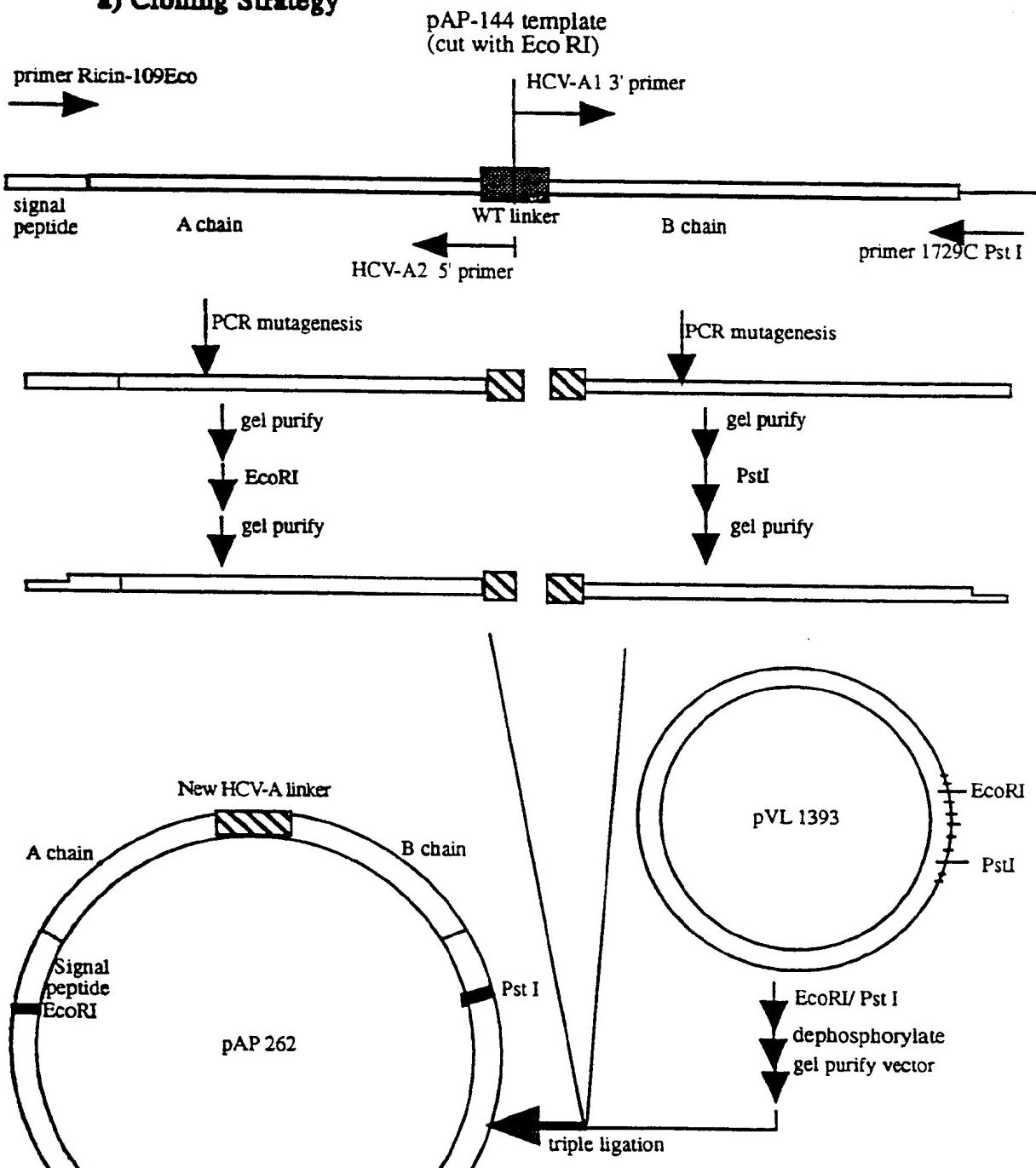
pAP-263/264 linker (CAP-C) :

A chain- S K P A E F F A L N F N -B chain

126/254

**FIGURE 29****PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

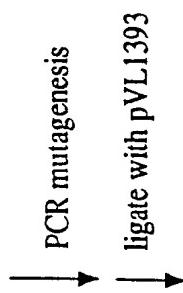
127/254

**FIGURE 30A****PCR Mutagenesis of Preproricin Gene to Create An HCV-A Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

128 / 254

**FIGURE 30B****Sequence of HCV-A Linker Region****WT prepricin linker****primer HCV-A1**

5' - TCGACATGGGTTTAATGCTGATGTT -3'  
 \* \* \* \* \* \* \* \*  
 TCTTGGCTATAAGGCCAGGGTGCCTAAATTAAAT  
 AGAAACGAATATCCGGTACACCGTTAAATAA  
 \* \* \* \* \* \* \* \*  
 3' - GGTAGCAGTGTCAAACTAACCTCCATCACTGTT 5'

**5' primer HCV-A2****pAP 262 linker  
(HCV-A variant)**

GATTTGAGGTAGTGACATGGGTTTAAT  
 CTAACCTCCATCACTGTAGCTACCCAAAATTA

129/254

**FIGURE 30C (P1)**

Sequence of pAP262 insert

10	20	30	40	50
1 GAATT CATGAAACCGGGAGGAAATACATTGTAATATGGATGTATGCAGT				
CTTAAGTACTTTGCCCTCCTTATGATAACATTACCTACATACGTCA				
51 GGCAACATGGCTTGTGATCCACCTCAGGGTGGCTTCACATTAG				
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAA				
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTACCA				
TCCTATTGTTGTATAAGGGTTGTTATGGTTAATATTGAAATGGTGT				
151 GCGGGTGCCACTGTGCAAAGCTACACAAACCTTATCAGAGCTGTCGCGG				
CGCCCACGGTGACACGTTGATGTGTTGAATAGTCTGACAAAGCGCC				
201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCAAGTGTGCCAA				
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAAACGGTT				
251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACCTCTCA				
TGTCTCAACCAAACGGATATTGGTTGCCAATAAAATCAACTTGAGAGT				
301 AATCATGCAGAGCTTCTGTACATTAGCGCTGGATGTACCAATGCATA				
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT				
351 TGTGGTCGGCTACCGTGCTGAAATAGCGCATATTCTTCATCCTGACA				
ACACCAGCCGATGGCACGACCTTATCGGTATAAGAAAGTAGGACTGT				
401 ATCAGGAAGATGCAGAAGCAATCACTCATTTTCACTGATGTTAAAAT				
TAGTCCTTCTACGTCTCGTTAGTAGAAAGTAGACTACAAGTTTA				
451 CGATATACTCGCTTGGTGGTAATTATGATAGACTTGAACAACTTGC				
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACCTGTTGAACG				
501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG				
ACCATTAGACTCTCTTATAGCTCAACCCTTACCAAGGTGATCTCCCTCC				
551 CTATCTCAGCGCTTATTACAGTACTGGTGGCACTCAGCTTCCAAC				
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA				
601 CTGGCTCGTTCTTATAATTGCATCCAAATGATTCAAGCAGCAAG				
GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTTCGTCGTT				
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA				
TAAGGTTATATAACTCCCTTTACGCGTCTTAATCCATGTTGGCCT				
701 GATCTGCACCAAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA				
CTAGACGTGGTCTAGGATCGCATTAAATGTGAACCTTATCAACCCCTCT				
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT				
GAAAGGTGACGTTAAGTCTCAGATTGGTCCTCGAACGATCAGGTTA				

130/254

**FIGURE 30C (P2)**

801 TCAACTGCAAAGACGTAATGGTCCAAATTCACTGTGTACGATGTGAGTA  
     AGTTGACGTTCTGCATTACCAAGGTTAACGTACACATGCTACACTCAT  
 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCCACCTCCACCA  
     ATAATTAGGGATAGTATCGAGAGTACCCACATATCTACGCGTGGAGGTGGT  
 901 TCGTCACAGTTGATGGAGGTAGTGACATCGACATGGGTTTTAATGC  
     AGCAGTGTCAAACAACTAACCTCCATCACTGTAGCTGTACCCAAAAATTACG  
 951 TGATGTTGTATGGATCCTGAGCCCAGTAGTGCCTATCGTAGGTGAAATG  
     ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
     CAGATACACAACATACAATCCCTACCTCTAAGGTGTTGCCCTTGCCTTAT  
 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
     GTCAACACCAGGTACGTTAGATTATGTCTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
     CTTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA  
     CCATGTCAGGCCCTCAGATACTACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCACATCATAAATCC  
     TGACTACGGTGGCGACCGTTACCTTACCTTGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
     GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTACCATGGTGTG  
 1301 TTACAGTGCACCAACACATTATGCCGTTAGTCAGGTTGGCTTCTACT  
     AATGTCACGTTGGTTGTAATAACGGCAATCAGTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
     TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAA  
     GAACGTTCGTTATCACCTGTTACACCTATCTCCTGACATCGTCACCTT  
 1451 AGGCTGAACAAACAGTGGCTTTATGCAGATGGTCAATACTGCTCAG  
     TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGAAACAGT  
     GTTTGGCTCTATTAACGGAATGTTCACTAAGATTATGCCCTTGTCA  
 1551 TGTAAAGATCCTCTTGTGGCCCTGCATCCTCTGGCAACGATGGATGT  
     ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGGTGTACCTACA  
 1601 TCAAGAATGATGGAACCATTAAATTGTATAGTGGATTGGTGTAGAT  
     AGTTCTTACTACCTTGGTAAAATTAAACATATCACCTAACCAACATCTA

131/254

**FIGURE 30C (P3)**

1651 GTGAGGCATCGGATCCGAGCCTAACAAATCATTCTTACCCCTCTCCA  
CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
1701 TGGTGACCAAACCAAATATGGTTACCATTATTTGATAGACAGATTACT  
ACCACTGGGTTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA  
1751 CTCTTGCAGTGTGTGTCCCTGCCATGAAAATAGATGGCTTAAATAAAAAA  
GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATTTT  
1801 GGACATTGTAATTTGTAACGTAAAGGACAGCAAGTTATATCGAATTCC  
CCTGTAACATTAAAACATTGACTTCCTGTCGTTCAATATAGCTTAAGG  
1851 TGCAG  
ACGTC

Total number of bases is: 1855.

Sequence name: PAP262

132/254

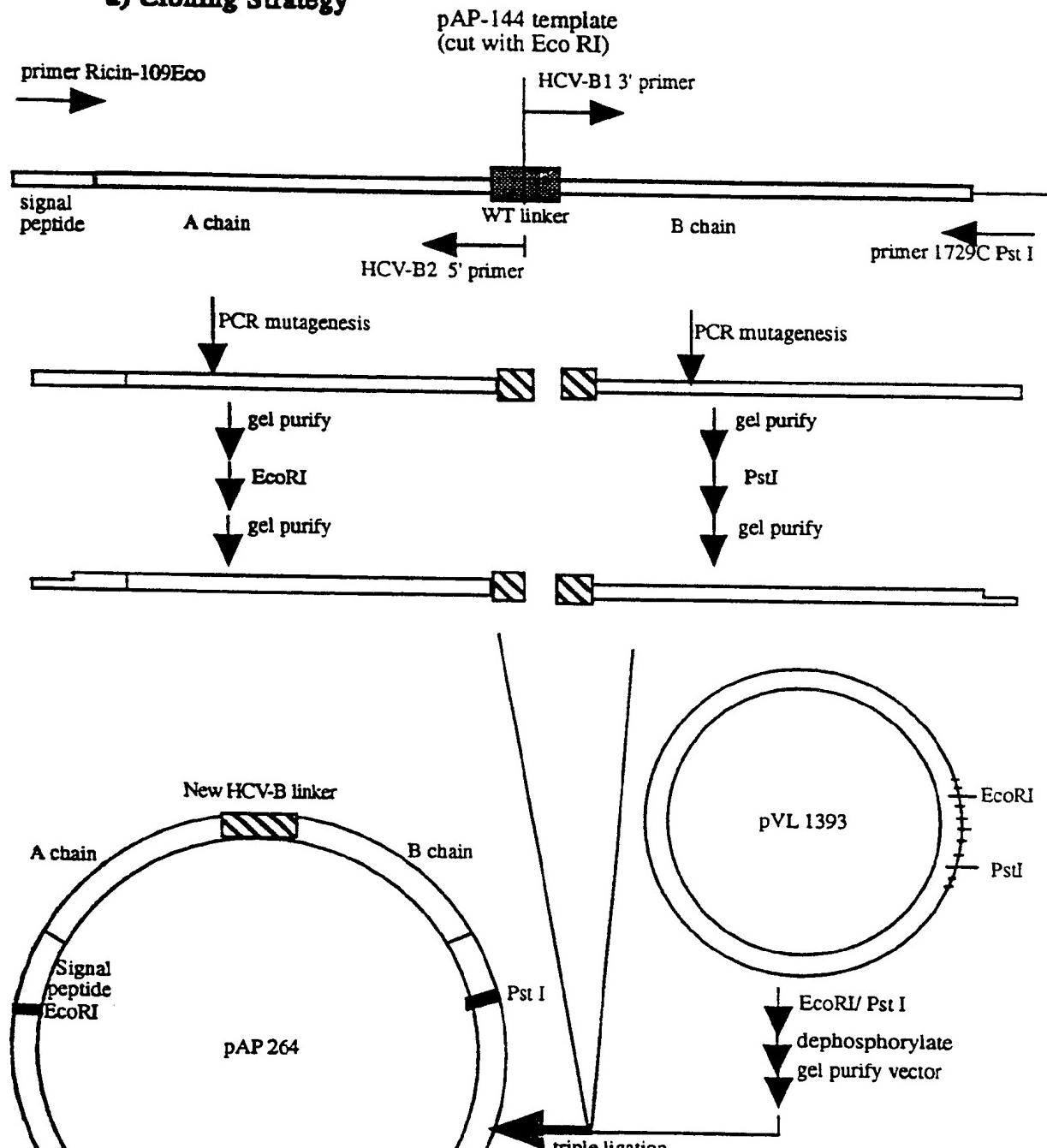
**FIGURE 30D**

**-Amino Acid Sequence Comparison of Mutant  
Preproricin Linker Region of HCV-A to Wild Type**

Wild type Ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-262 linker: (HCV-A linker) A chain- D L E V V T S T W V F N -B chain

133/254

**FIGURE 31A****PCR Mutagenesis of Preproricin Gene to Create An HCV-B Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

134/254

**FIGURE 31B**

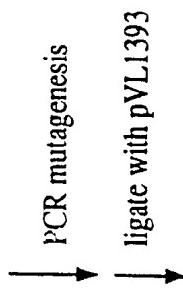
**Sequence of HCV-B Linker Region**

**WT preproline linker**

**primer HCV-B1**

5' - GCGTCACACCTTTAATGCTGATGTT -3'  
 \* \* \* \* \* \* \* \*  
 TCTTTCGCTTATAAGGCCAGTGGTGCCTAAATTAAAT  
 AGAACGGAATATTCCGGTCAACCACGGTTAAATTA  
 \* \* \* \* \* \* \* \*  
 3' - GGTAGCAGTGTCAAACTACTTACCTTCTCACCA -5'

**5' primer HCV-B2**



**pAP 264 linker  
(HCV-B variant)**

GATGAGATGGAAGAGTGTGGCTCACACCTTTAAT  
 CTACTCTACCTTCTCACACGCAGTGTGGAAAAATTA

135/254

**FIGURE 31C (P1)**

Sequence of pAP264 insert

10	20	30	40	50
1 GAATT CATGAAACCGGGAGGAATACTATTGTAATATGGATGTATGCAGT CTTAAGTACTTGGCCCTCCTTATGATAACATTACCTACATACGTCA				
51 GGCAACATGGCTTGTTGGATCCACCTCAGGGTGGCTTCACATTAG CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC				
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAAGTTACCA TCCTATTGTTGATAAGGGTTGTTATGGGTAATATTGAAATGGTGT				
151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTTCGGG CGCCCACGGTACACGTTGATGTGTTGAAATAGTCTGACAAGCGCC				
201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTGCAA AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT				
251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACTCTCA TGTCTAACCAAACGGATATTGGTTGCAAATAAAATCAACTTGAGAGT				
301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT				
351 TGTGGTCGGCTACCGTGGAAATAGCGCATATTCTTCATCCTGACA ACACCAGCCGATGGCACGACCTTATCGCGTATAAGAAAGTAGGACTGT				
401 ATCAGGAAGATGCAGAAGCAACTCACTCATCTTCACTGATGTTCAAAT TAGTCCTCTACGTCTCGTTAGTAGAGAAAAGTGAACAGTTTA				
451 CGATATACATTGCCTTGTTGGTAATTATGATAGACTGAACAACTTGC GCTATATGTAAGCGAAACCACCATTAATACTATCTGAACCTGTTGAACG				
501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG ACCATTAGACTCTTTATAGCTAACCCCTTACCAAGGTGATCTCCTCC				
551 CTATCTCAGCGCTTATTACAGTACTGGTGGCACTCAGCTTCAA GATAGAGTCGCGAAATAATGTACGTTACTAAAGTCTCGTGTGAAAGGTTGA				
601 CTGGCTCGTTCTTATAATTGACATCCAAATGATTTCAGAACGAGCAAG GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTGTTC				
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA TAAGGTTATATAACTCCCTCTTACCGTGTCTTAATCCATGTTGGCCT				
701 GATCTGACCCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA CTAGACGTGGTCTAGGATCGCATTAAATGTGAACCTTATCAACCCCTCT				
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCAA GAAAGGTGACGTTAAGTCTCAGATTGGTCTCGAAACGATCAGGTTA				

136/254

**FIGURE 31C (P2)**

801 TCAACTGCAAAGACGTAATGGTCCAATTCACTGTGTACGATGTGAGTA  
     AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT  
 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA  
     ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT  
 901 TCGTCACAGTTGATGAGATGGAAGAGTGTGCGTCACACCTTTAATGC  
     AGCAGTGTCAAACACTACTCTACCTCTCACACGCAGTGTGGAAAAATTACG  
 951 TGATGTTGTATGGATCCTGAGCCATAGTGCCTATCGTAGGTCGAAATG  
     ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATCCACAACGGAAACGCAATA  
     CAGATACACAACATACCTACCCCTACCTCTAAGGTGTGCCTTGCCTTAC  
 1051 CAGTTGTGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
     GTCAACACCGGTACGTTAGATTATGTCTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
     CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA  
     CCATGTCAGGCCCTCAGATACTACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGATAATGGAACCATCATAATCC  
     TGACTACGGTGGCGACCCTTATACCCTATTACCTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTAGCAGCGACATCAGGAAACAGTGGTACCAACAC  
     GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTCACCATGGTGTG  
 1301 TTACAGTGCAAACCAACATTATGCCGTTAGTCAGGTTGGCTTCTACT  
     AATGTCACGTTGGTTGTAATACGGCAATCAGTCCAACCGAAGGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
     TTATTATGTGTTGGAAACAATGTTGGTAACAACCCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
     GAACGTTCGTTATCACCTGTTACACCTATCTCCTGACATCGTCACTTT  
 1451 AGGCTGAACAACAGTGGGCTCTTATGCAGATGGTCAATACGTCTCAG  
     TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGTCTAATATACGGAAACAGT  
     GTTTGGCTCTATTAACGGAATGTTACTAAGATTATGCCCTTGTCA  
 1551 TGTAAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
     ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA  
 1601 TCAAGAATGATGGAACCATTAAATTGTATAGTGGATTGGTAGAT  
     AGTTCTTACTACCTGGTAAAATTAAACATATCACCTAACCAATCTA

137/254

**FIGURE 31C (P3)**

1651 GTGAGGCGATCGGATCCGAGCCTAAACAAATCATTCTTACCCCTCTCCA  
CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTGATAGACAGATTACT  
ACCACTGGGTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGCCTGCCATGAAAATAGATGGCTTAAATAAAA  
GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATTTT

1801 GGACATTGTAATTTGTAAGTGAAGGACAGCAAGTTATATCGAATTCC  
CCTGTAACATTAAAACATTGACTTCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG  
ACGTC

Total number of bases is: 1855.

Sequence name: PAP264

138/254

**FIGURE 31D**

**-Amino Acid Sequence Comparison of Mutant  
Preproricin Linker Region of HCV-B to Wild Type**

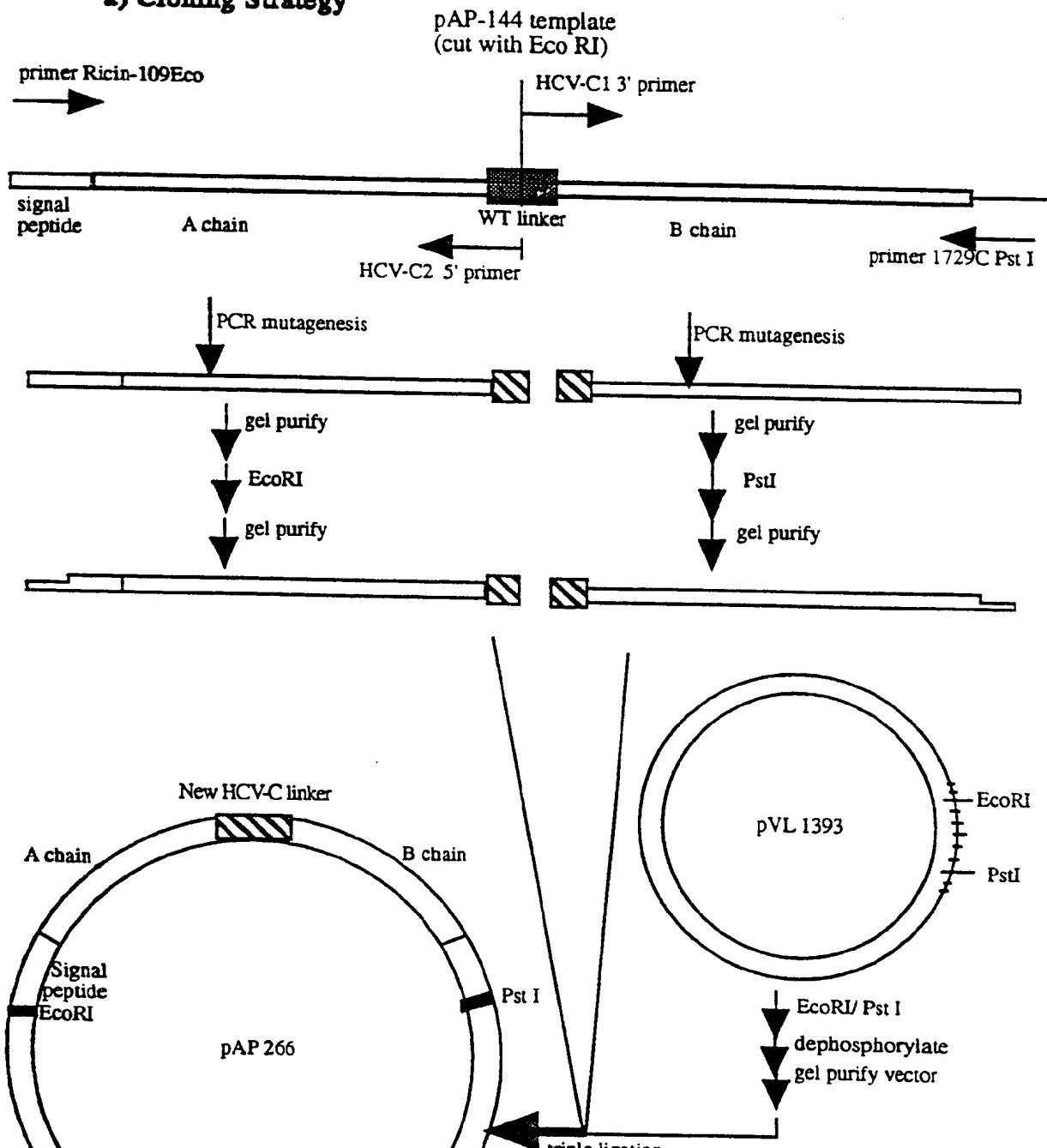
Wild type Ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-264 linker:  
(HCV-B linker)                    A chain- D E M E E C A S H L F N -B chain

139/254

**FIGURE 32A**

- PCR Mutagenesis of Preproricin Gene to Create An HCV-C Variant Gene in Baculovirus Transfer Vector, pVL 1393

**a) Cloning Strategy**

**FIGURE 32B**

## Sequence of HCV-C Linker Region

WT preprorcin linker

primer HCV-C1

5' primer HCV-C2

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graph TD
    A[PCR mutagenesis] --> B["ligate with pVL1393"]

```

pAP266 linker  
(HCV-C variant)

-GAGGACGTTGTATGTTGTCATATTAACTCCTGCAAACATACAAAGCTACAGTAAAAATT-

141/254

**FIGURE 32C (P1)**

Sequence of pAP266 insert

10	20	30	40	50
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT CTTAAGTACTTTGCCCTCTTATGATAACATTACCTACATACGTCA				
51 GGCACACATGGCTTGTTGGATCCACCTCAGGGTGGCTTCACATTAG CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAA				
101 AGGATAACAACATATTCCCCAACAAATACCCAATTATAAACTTACCA TCCTATTGTTGATAAGGGTTGTTATGGGTTAATATTGAAATGGTGT				
151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTTGCC CGCCCACGGTGACACGTTGATGTGTTGAAATAGTCTCGACAAGCGCC				
201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTGCC AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGT				
251 ACAGAGTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACCTCA TGTCTAACCAAACGGATATGGTGCCTAACAAATCAACTTGAGAGT				
301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA TTAGTACGTCTCGAAAGACAATGTAATCGCACCTACAGTGGTTACGTAT				
351 TGTGGTCGGCTACCGTGTGGAAATAGCGCATATTCTTCATCCTGACA ACACCAGCCGATGGCACGACCTTATCGGTATAAGAAAAGTAGGACTGT				
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTCACTGATGTTCAAAAT TAGTCCTCTACGTCTCGTTAGTAGAAAGTAGACTACAAGTTTA				
451 CGATATACATTCGCCTTGGTGGTAATTATGATAGACTTGAACAACTTGC GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACCTGTTGAACG				
501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG ACCATTAGACTCTCTTATAGCTAACCCCTTACCAAGGTGATCTCCTCC				
551 CTATCTAGCGCTTTATTACAGTACTGGTGGCACTCAGCTTCCA GATAGAGTCGCGAAATAATAATGTATGACCGTGTGAGTCGAAGGTTGA				
601 CTGGCTCGTCCTTATAATTGCATCCAAATGATTCAGAAGCAGCAAG GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTCGTT				
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA TAAGGTTATATAACTCCCTCTTACCGCGTGTCTTAATCCATGTTGCC				
701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA CTAGACGTGGTCTAGGATCGCATTATGTGAACCTTATCAACCCCTCT				
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCA GAAAGGTGACGTTAAGTTCTCAGATTGGTCTCGGAAACGATCAGGTTA				

142/254

**FIGURE 32C (P2)**

801 TCAACTGCAAAGACGTAATGGTCCAAATTCACTGTGTACGATGTGAGTA  
 AGTTGACGTTCTGCATTACCAAGGTTAACGTACACACATGCTACACTCAT  
 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCCACCTCCACCA  
 ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACGCGTGGAGGTGGT  
 901 TCGTCACAGTTGAGGACGTTGTATGTTGTCATGTCATATTTAATGC  
 AGCAGTGTCAAACCTCTGCAACATACAACAAGCTACAGTATAAAATTACG  
 951 TGATGTTGTATGGATCCTGAGCCCATACTGCGTATCGTAGGTGAAATG  
 ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
 CAGATACACAACATACAATCCCTACCTCTAAGGTGTTGCCCTTGCCTTAT  
 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTCACTGATTATGTCTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
 CTTTCCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA  
 CCATGTCAGGCCCTCAGATACTACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCACATCATAAATCC  
 TGACTACGGTGGCGACCCTTATACCTTATTACCTTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
 GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTCAACCATGGTGTG  
 1301 TTACAGTGCAAACCAACATTATGCCGTTAGTCAGGTTGGCTTCTACT  
 AATGTCACGTTGGTTGTAATACGGCAATCAGTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGCTGTG  
 TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
 GAACGTTGTTATCACCTGTTACACCTATCTCTGACATCGTCACTTT  
 1451 AGGCTGAACAAACAGTGGCTCTTATGCAGATGGTCAATACGTCCTCAG  
 TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGATTCTAATATACGGGAAACAGT  
 GTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTGTCA  
 1551 TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
 ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGGTGTACCTACA  
 1601 TCAAGAATGATGGAACCATTAAATTGTATAGTGGATTGGTAGAT  
 AGTTCTTACCTTGGTAAAATTAAACATATCACCTAACCAACATCTA

143/254

**FIGURE 32C (P3)**

1651 GTGAGGCATCGATCCGAGCCTAAACAAATCATTCTTACCCCTCTCCA  
CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTGATAGACAGATTACT  
ACCACTGGGTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA

1751 CTCTGCAGTGTGTGTGCCTGCCATGAAAATAGATGGCTAAATAAAAAA  
GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATTTT

1801 GGACATTGTAATTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC  
CCTGTAACATTAAAACATTGACTTCCGTCAATATAGCTTAAGG

1851 TGCAG  
ACGTC

Total number of bases is: 1855.

Sequence name: PAP266

144/254

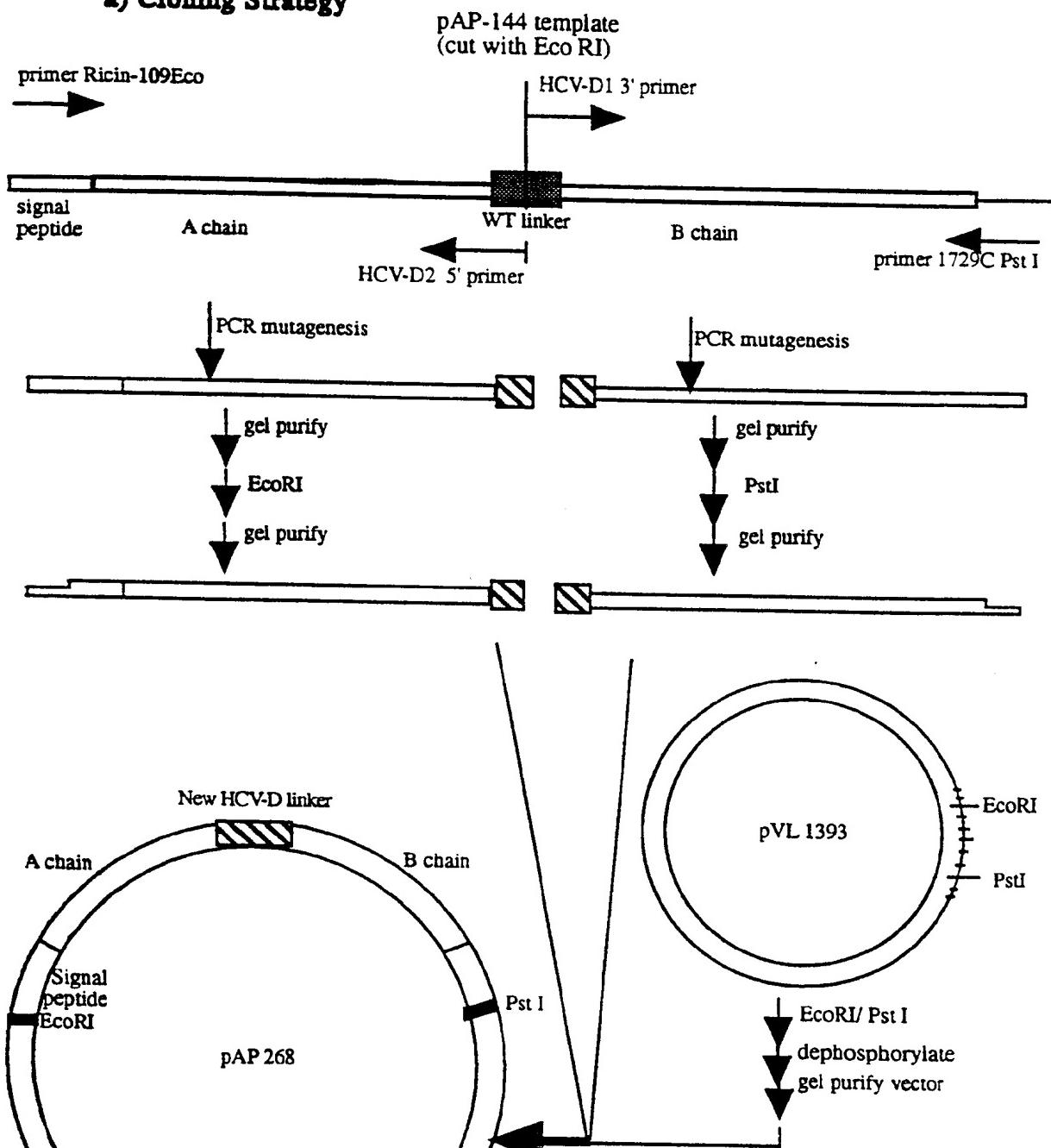
**FIGURE 32D**

**-Amino Acid Sequence Comparison of Mutant  
Preproricin Linker Region of HCV-C to Wild Type**

Wild type Ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-266 linker:  
(HCV-C linker)                    A chain- E D V V C C S M S Y F N -B chain

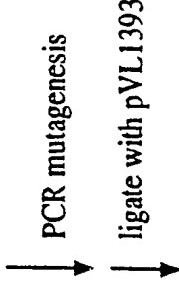
145/254

**FIGURE 33A****PCR Mutagenesis of Preproricin Gene to Create An HCV-D Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

146/254

**FIGURE 33B****Sequence of HCV-D Linker Region****WT preprotein linker****primer HCV-D1**

5' - GCGCCAATAACTGCTTATGGTGTATG - 3'  
 \*  
 TCTTTGCTTATAAGGCCAAGTGGTGCCTAAATTAAAT  
 AGAAACGAATATTCCGGTACCCACGGTTAAATTA  
 \*  
 3' - GGTAGCAGTGTCAAATCCCCACCTCTAACGAT - 5'

**5' primer HCV-D2****pAP 268 linker  
(HCV-D variant)**

AAGGGCTGAGATTGCTAGGCCAATAACTGCTTAT  
TCCCCACCTCTAACGATCGGGTTATGACGAATA

147/254

**FIGURE 33C (P1)**

Sequence of pAP268 insert

10	20	30	40	50
1 GAATTCATGAAACCGGGAGGAAATACTATTGTAAATATGGATGTATGCAGT				
CTTAAGTACTTGGCCCTCCTTATGATAACATTATACCTACATACGTCA				
51 GGCAACATGGCTTGTGATCCACCTCAGGGTGGCTTCACATTAG				
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC				
101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA				
TCCTATTGTTGTATAAGGGTTGTATGGTTAATATTGAAATGGTGT				
151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTTCGCGG				
CGCCCACGGTGACACGTTGATGTGTTGAAATAGTCTCGACAAAGCGCC				
201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTGCCAA				
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT				
251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACCTCTCA				
TGTCTCAACCAACGGATATTGGTTGCCAAATAAAATCAACTTGAGAGT				
301 AATCATGCAGAGCTTCTGTACATTAGCGCTGGATGTACCAATGCATA				
TTAGTACGTCTCGAAAGACAATGTAATCGCACCTACAGTGGTTACGTAT				
351 TGTGGTCGGCTACCGTGCTGAAATAGCGCATATTCTTCATCCTGACA				
ACACCAGCCGATGGCACGACCTTATCGGTATAAGAAAGTAGGACTGT				
401 ATCAGGAAGATGCAGAACGAACTCACTCATTTTCACTGATGTTAAAAT				
TAGTCCTTCTACGTCTCGTTAGTGAGTAGAAAGTGAACACTAACAGTTTA				
451 CGATATACTCGCCTTGGGTAATTATGATAGACTTGAACAACTTGC				
GCTATATGTAAGCGAAACCACCATTAATACTATCTGAACCTGTTGAACG				
501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG				
ACCATTAGACTCTCTTATAGCTCAACCCTTACCAAGGTGATCTCCTCC				
551 CTATCTCAGCGCTTATTACAGTACTGGTGGCACTCAGCTTCAAAC				
GATAGAGTCGCGAAATAATAATGTATGACCACCGTGAGTCGAAGGGTGA				
601 CTGGCTCGTCTTATAATTGCATCCAAATGATTCAGAACAGCAAG				
GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTGTTC				
651 ATTCCAATATATTGAGGGAGAAATGCCACGAGAATTAGGTACAACCGGA				
TAAGGTTATATAACTCCCTCTTACCGTGCTCTTAATCCATGTTGGCCT				
701 GATCTGCACCAAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA				
CTAGACGTGGTCTAGGATCGCATTAAATGTAACTCTTATCAACCCCTCT				
751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT				
GAAAGGTGACGTTAAGTTCTCAGATTGGTCTCGGAAACGATCAGGTTA				

148/254

**FIGURE 33C (P2)**

801 TCAACTGCAAAGACGTAATGGTCCAAATTCACTGTGTACGATGTGAGTA  
 AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT  
 851 TATTAATCCCTATCATAGCTCATGGGTATAGATGCGCACCTCCACCA  
 ATAATTAGGGATAGTATCGAGAGTACACATATCTACGCGTGGAGGTGGT  
 901 TCGTCACAGTTAACGGGTGGAGATTGCTAGGCCAATAACTGCTTATGC  
 AGCAGTGTCAAATTCCCCACCTCTAACGATCGCGTTATTGACGAATACG  
 951 TGATGTTGTATGGATCCTGAGCCCAGTAGTGCATCGTAGGTCGAAATG  
 ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGAAACGCAATA  
 CAGATACACAACATACAATCCCTACCTTCTAACGGTTAGTGCCTTGCGTTAT  
 1051 CAGTTGTGCCATGCAAGTCTAACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTAGATTATGTCAGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTCGATCTAACGGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAAAACTGCTGCA  
 CCATGTCAGGCCCTCAGATAACTACTAGATAACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCACATCATAAATCC  
 TGACTACGGTGGCGACCGTTATACCCATTACCTTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTAGCAGCGACATCAGGAAACAGTGGTACCAACAC  
 GTCTAGATCAGATCAAATCGCGTGTAGTCCCTGTCACCATGGTGTG  
 1301 TTACAGTGCACCAACATTTATGCCGTTAGTCAGGTTGGCTTCAACT  
 AATGTCACGTTGGTTGTAATACGGCAATCAGTCCAAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
 TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGGACTGTAGCAGTGAAA  
 GAACGTTCGTTATCACCTGTCATACCTATCTCCTGACATCGTCACTTT  
 1451 AGGCTGAACAAACAGTGGCTCTTATGCAGATGGTCAATACGTCCTCAG  
 TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGATTCTAACGGAAACAGT  
 GTTTGGCTCTATTAACGGAATGTTCACTAACGATTATATGCCCTTGTCA  
 1551 TGTAAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
 ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTAC  
 1601 TCAAGAATGATGGAACCATTAAATTGTATAGTGGATTGGTAGAT  
 AGTTCTTACTACCTTGGTAAATTAACATATCACCTAACCAACATCTA

149/254

**FIGURE 33C (P3)**

1651 GTGAGGCATCGGATCCGAGCCTAAACAAATCATTCTTACCCCTCCA  
CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCAAACCAAATATGGTACCATATTGATAGACAGATTACT  
ACCACTGGGTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGCCTGCCATGAAAATAGATGGCTTAAATAAAAA  
GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATT

1801 GGACATTGTAATTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC  
CCTGTAACATTAAAACATTGACTTCCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG  
ACGTC

Total number of bases is: 1855.

Sequence name: PAP268

150/254

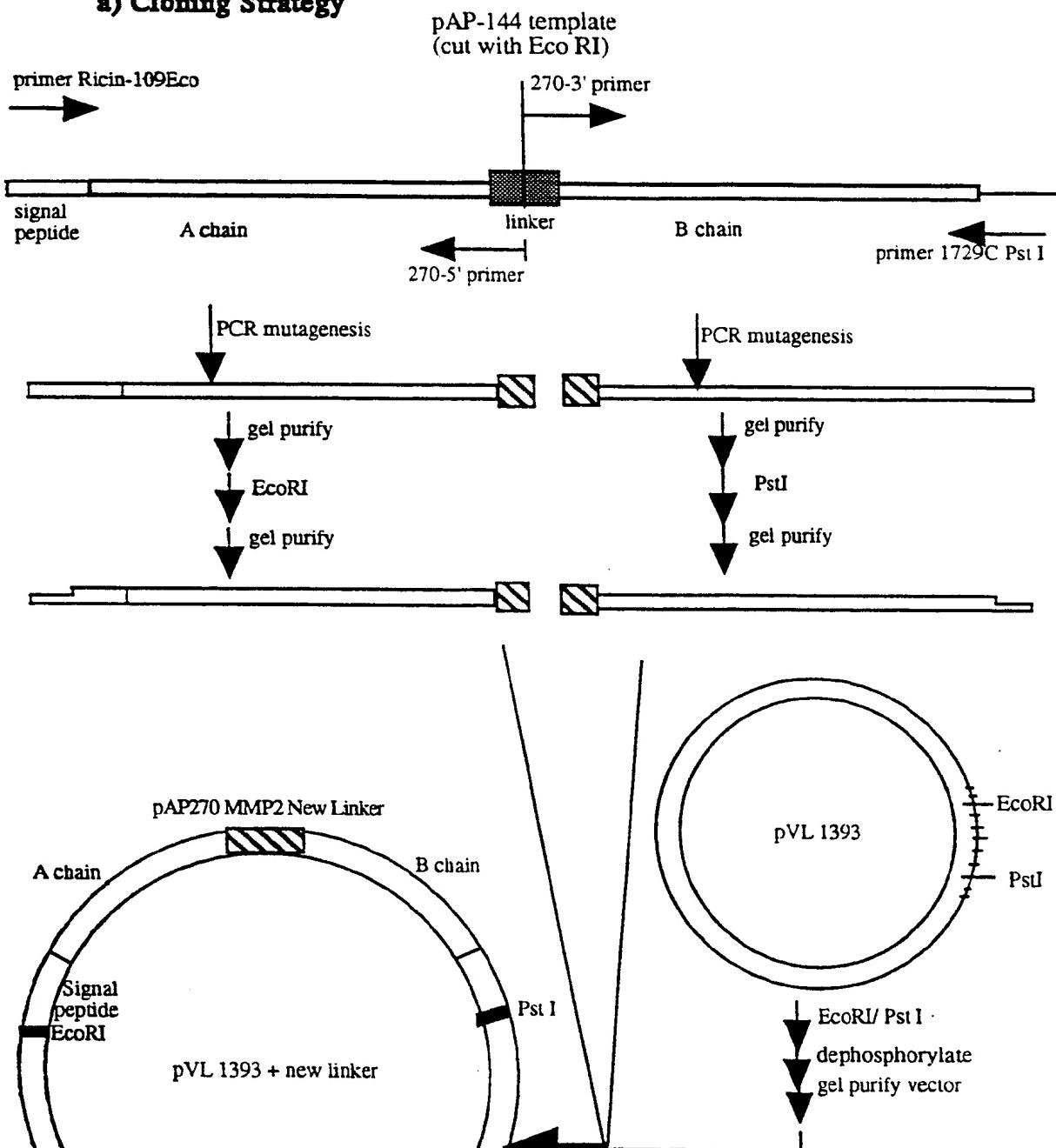
**FIGURE 33D**

**-Amino Acid Sequence Comparison of Mutant  
Preproricin Linker Region of HCV-D to Wild Type**

Wild type Ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-268 linker:  
(HCV-D linker)                    A chain- K G W R L L A P I T A Y -B chain

151/254

**FIGURE 34A****PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

152/254

**FIGURE 34B****Sequence of MMP-2 Linker Region****WT preprocin linker**

primer 270-3'  
 5' - TGGGCTCCTAATTTAATGCTGATGTTGT -3'  
 | \*\* \* \* \*  
 -----TCTTTGCTTATAAGGCCA| GTGGTACCAAATTTAAT-----  
 -----AGAAACGAATATTCCGGT| CACCATGGTTAAAATTA-----  
 \*\*\* \* \* \*\*\*  
 3' - AGCAGTGTCAAAAGAAACGGGGACCCAAAT -5'  
 primer 270-5'

1) PCR mutagenesis

2) Ligate with pVL1393

**pAP 270 linker  
(MMP-2 variant)**

-----TCTTTGCCCTGGGTTA| TGGGCTCCTAATTTAAT-----  
 -----AGAAACGGGGACCCAAAT| ACCCGAGGGATTAAAATTA -----

153/254

**FIGURE 34C (P1)**

Sequence of pAP270 insert

1	10 	20 	30 	40 	50 
---	--------	--------	--------	--------	--------

GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT  
 CTTAAGTACTTGGCCCTCCTTATGATAACATTATACTACATACGTCA  
  
 51 GGCAACATGGCTTGTGATCCACCTCAGGGTGGTCTTCACATTAG  
 CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC  
  
 101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCA  
 TCCTATTGTTGTATAAGGGTTGTTATGGTTAATATTGAAATGGTGT  
  
 151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTCGG  
 CGCCCACGGTGACACGTTCGATGTGTTGAAATAGTCTCGACAAGCGCC  
  
 201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGGCCAA  
 AGCAAATTGTTGACCTCGACTACACTCTGTACTATGGTCACAACGGTT  
  
 251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACCTCA  
 TGTCTAACCAACGGATATTGGTTGCCAAATAAAATCAACTTGAGAGT  
  
 301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA  
 TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT  
  
 351 TGTGGTCGGCTACCGTGGAAATAGCGCATATTCCTTCATCCTGACA  
 ACACCAGCCGATGGCACGACCTTATCGCGTATAAGAAAGTAGGACTGT  
  
 401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTCACTGATGTTAAAAT  
 TAGTCCTTCTACGTCTCGTTAGTAGAAAAGTGAACACTACAAGTTTA  
  
 451 CGATATACATTGCCCTTGGTGGTAATTATGATAGACTTGAACAACTTGC  
 GCTATATGTAAGCGGAAACCACCATTAATAACTATCTGAACCTGTTGAACG  
  
 501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG  
 ACCATTAGACTCTCTTATAGCTAACCTTACCAAGGTGATCTCCTCC  
  
 551 CTATCTAGCGCTTTATTACAGTACTGGTGGCACTCAGCTTCAACT  
 GATAGAGTCGCGAAATAATAATGTCATGACCAACCGTGAGTCGAAGGTTGA  
  
 601 CTGGCTCGTCTTATAATTGCATCCAAATGATTCAAGCAGCAAG  
 GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTCGTT  
  
 651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA  
 TAAGGTTATATAACTCCCTTTACCGCGTCTTAA~~TGCA~~~~T~~TTGGCCT

154/254

**FIGURE 34C (P2)**

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA  
 CTAGACGTGGTCTAGGATCGCATTAAATGTGAACCTTATCAACCCCTCT  
  
 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT  
 GAAAGGTGACGTTAAGTTCTCAGATTGGTCCTCGGAAACGATCAGGTTA  
  
 801 TCAACTGCAAAGACGTAATGGTCCAAATTCAAGTGTACGATGTGAGTA  
 AGTTGACGTTCTGCATTACCAAGGTTAACGACACATGCTACACTCAT  
  
 851 TATTAATCCCTATCATAGCTCTCATGGTGTAGATGCCACCTCCACCA  
 ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACCGTGGAGGTGGT  
  
 901 TCGTCACAGTTTCTTGCCCCCTGGGTTATGGGCTCTAATTTAATGC  
 AGCAGTGTCAAAAGAACGGGACCCAAATACCCGAGGATTAAAATTACG  
  
 951 TGATGTTGTATGGATCCTGAGCCCAGTGCCTAGTAGGTCGAAATG  
 ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
 CAGATAACACAACATACTACCCCTACCTTCTAACGGTTGTTGCCTTGCATT  
  
 1051 CAGTTGTGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGTACGTTAGATTATGTCAGTTAGTCGAGACCTGAAA  
  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAAACTGCTGCA  
 CCATGTCAGGCCCTCAGATAACACTACTAGATACTAACGTTATGACGACGT  
  
 1201 ACTGATGCCACCCGCTGGCAAATATGGATAATGGAACCACATCAAATCC  
 TGACTACGGTGGCGACCGTTATACCCATTACCTTGGTAGTATTTAGG  
  
 1251 CAGATCTAGTCTAGTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
 GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTCACCATGGTGTG  
  
 1301 TTACAGTGCACCAACACATTATGCCGTTAGTCAAGGTTGGCTCCTACT  
 AATGTCACGTTGGTTGTAACACGGCAATCAGTTCCAACCGAAGGATGA  
  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
 TTATTATGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC  
  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
 GAACGTTCGTTATCACCTGTTACCTATCTCCTGACATCGTCACTT

155/254

**FIGURE 34C (P3)**

1451 AGGCTGAACAAACAGTGGCTTTATGCAGATGGTCAATAACGTCCCTCAG  
TCCGACTTGTGTCACCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATAACGGAAACAGT  
GTTTGGCTCTATTACCGAATGTTCACTAAGATTATGCCCTTGTCA

1551 TGTAAAGATCCTCTCTGTGGCCCTGCATCCTCTGCCAACGATGGATGT  
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCCTGCTACCTACA

1601 TCAAGAAATGATGGAACCATTTAAATTGTATAGTGGATTGGTAGAT  
AGTTCTTACTACCTTGGTAAAATTAAACATATCACCTAACCAACATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTACCCCTCTCCA  
CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTGATAGACAGATTACT  
ACCACTGGGTTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAAAAA  
GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATTTT

1801 GGACATTGTAATTTGTAAGTGAAAGGACAGCAAGTTATATCGAATTCC  
CCTGTAACATTAAACATTGACTTCCGTCAATATAGCTTAAGG

1851 TGCAG  
ACGTC

Total number of bases is: 1855.

Sequence name: PAP270

156/254

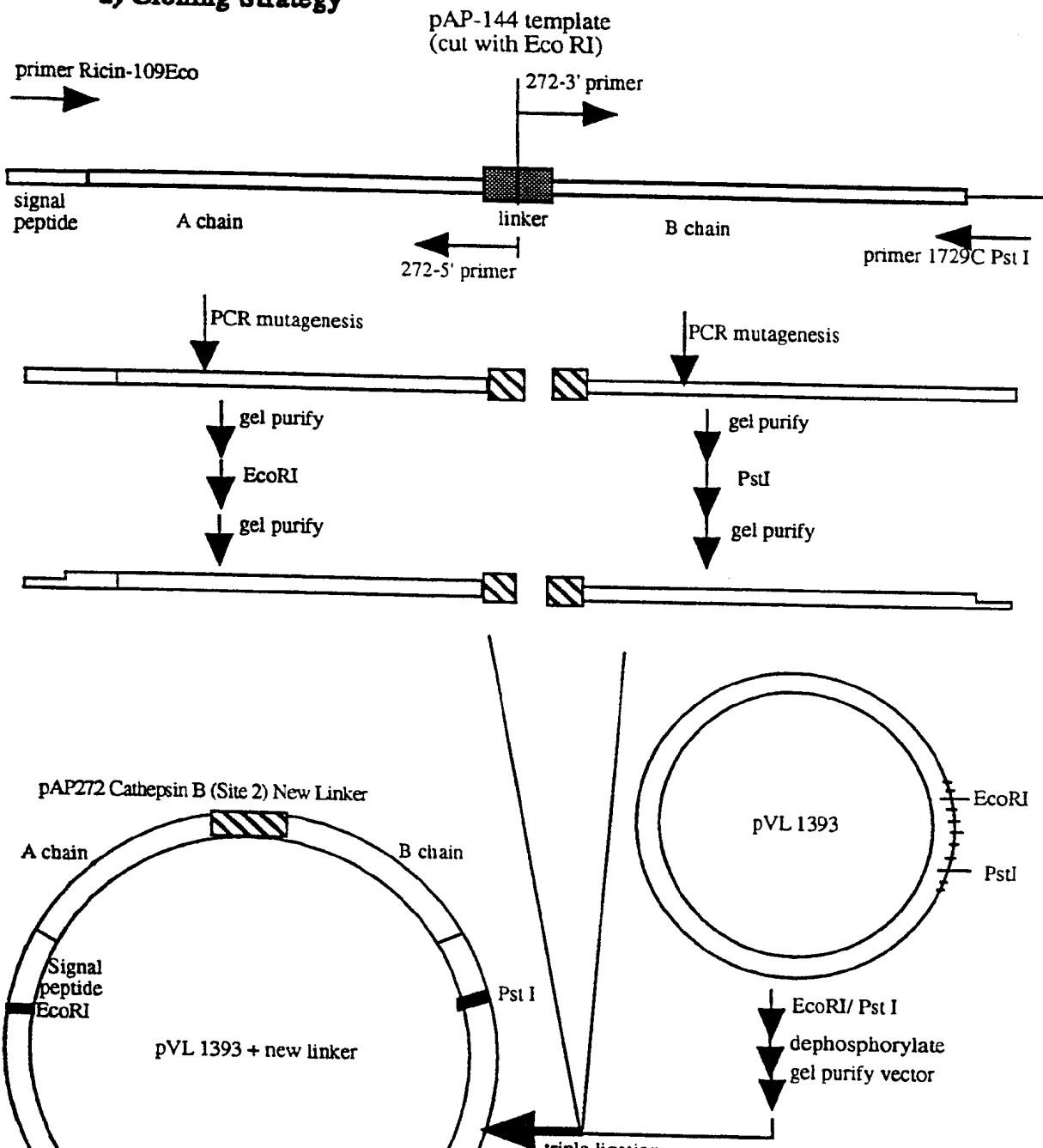
**FIGURE 34D**

**Amino acid sequence Comparison of Mutant Preproricin Linker  
region of MMP-2 to Wild Type**

Wild type ricin linker:            A chain- S L L I R P V V P N F N -B chain

pAP-270 (MMP-2) linker:            A chain- S L P L G L W A P N F N -B chain

157/254

**FIGURE 35A****PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

158/254

**FIGURE 35B****Sequence of Cathepsin B (Site 2) Linker Region****WT preprocin linker**

primer 272-3'

5' - AGGATGCCAAATTAAATGCTGATGTTGT - 3'

| \* \* \* \*

----- TCTTTGCTTATAAGGCCA | GTGGTACCAAATTAAAT -----

----- AGAACGAATATCCGGT | CACCATGGTTAAAATTA -----

\*\*\*\*\*

3' - AGCAGTGTCAAAAGAACGAATATCGATCT - 5'

primer 272-5'

1) PCR mutagenesis

2) Ligate with pVL1393

**pAP 272 linker****(Cathepsin B Site 2 variant)**

----- TCTTTGCTTATAAGCTAGA | AGGATGCTAATTAAAT -----

----- AGAACGAATATCGATCT | TCCTACGGATTAAAATTA -----

159/254

**FIGURE 35C (P1)**

Sequence of pAP272 insert

10	20	30	40	50

1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT  
 CTTAAGTACTTGGCCCTCCTTATGATAACATTATAACCTACATACGTCA  
 51 GGCAACATGGCTTGTGATCCACCTCAGGGTGGCTTCACATTAG  
 CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC  
 101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA  
 TCCTATTGTTGTATAAGGGGTTGTTATGGGTTAATATTGAAATGGTGT  
 151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTTCGCGG  
 CGCCCACGGTGACACGTTCGATGTGTTGAAATAGTCTCGACAAGCGCC  
 201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATAACCAGTGGTGC  
 AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT  
 251 ACAGAGTTGGTTGCCCTATAAACCAACGGTTATTTAGTTGAACCTCTCA  
 TGTCTAACCAAACGGATATTGGTTGCCAAATAAAATCAACTTGAGAGT  
 301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA  
 TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT  
 351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTCTTCATCCTGACA  
 ACACCAGCCGATGGCACGACCTTATCGGTATAAAGAAAGTAGGACTGT  
 401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTCACTGATGTTCAAAAT  
 TAGTCCTTCTACGTCTCGTTAGTAGAAAGTGAACACTACAAGTTTA  
 451 CGATATACATTGCCCTTGGTGGTAATTATGATAGACTTGAACAAACTGC  
 GCTATATGTAAGCGGAAACCACCAATTAAACTATCTGAACCTGTTGAACG  
 501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG  
 ACCATTAGACTCTCTTTATAGCTCAACCCTTACCAAGGTGATCTCCTCC  
 551 CTATCTAGCGCTTATTACAGTACTGGTGGCACTCAGCTTCAACT  
 GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA  
 601 CTGGCTCGTTCTTATAATTGCATCCAAATGATTCAGAAGCAGCAAG  
 GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTCGTT  
 651 ATTCCAATATATTGAGGGAGAAATGCCACGAGAATTAGGTACAACCGGA  
 TAAGGTTATATAACTCCCTCTTACCGGTGCTTTAATCCATGTTGGCCT

160/254

**FIGURE 35C (P2)**

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA  
 CTAGACGTGGTAGGATCGCATTATGTGAACCTTATCAACCCCTCT  
 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT  
 GAAAGGTGACGTTAAGTCTCAGATTGGTCCTCGGAAACGATCAGGTTA  
 801 TCAACTGCAAAGACGTAATGGTCCAAATTCAAGTGTACGATGTGAGTA  
 AGTTGACGTTCTGCATTACCAAGGTTAACGTACACACATGCTACACTCAT  
 851 TATTAATCCTATCATAGCTCTCATGGTGTAGATGCGCACCTCCACCA  
 ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACGCGTGGAGGTGGT  
 901 TCGTCACAGTTCTTGCTTATAGCTAGAAGGATGCCTAATTAAATGC  
 AGCAGTGTCAAAAGAAAGGAATATCGATCTCCTACGGATTAAAATTACG  
 951 TGATGTTGTATGGATCCTGAGCCCAGTGCCTAGTAGTCGAAATG  
 ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
 CAGATAACACAACATACTACCCCTACCTCTAAGGTGTTGCCTTGCCTTAT  
 1051 CAGTTGTGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTCAGATTATGTCTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAAACTGCTGCA  
 CCATGTCAGGCCCTCAGATAACACTACTAGATAACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCGCTGGCAAATATGGATAATGGAACCATCATAAATCC  
 TGACTACGGTGGCGACCGTTATACCCATTACCTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
 GTCTAGATCAGATCAAATCGCGCTGTAGTCCCTGTCACCATGGTGTG  
 1301 TTACAGTGCAAACCAACATTATGCCGTTAGTCAGGTTGGCTTCAACT  
 AATGTCACGTTGGTTGAAATACGGAATCAGTTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCAATTGTTGGCTATATGGTCTGTG  
 TTATTATGTGTTGGAAAACAATGTTGTAACAACCCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
 GAACGTTCGTTATCACCTGTTACCTATCTCCTGACATCGTCACCTT

161/254

**FIGURE 35C (P3)**

1451 AGGCTGAACAAACAGTGGCTTTATGCAGATGGTCAATAACGTCTCAG  
TCCGACTTGTGTCACCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATAACGGAAACAGT  
GTTTGCGCTCTATTAACGGAATGTTCACTAAGATTATGCCCTTGTCA

1551 TGTAAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTAAATTGTATAGTGGATTGGTGTAGAT  
AGTTCTTACTACCTTGGTAAAATTAAACATATCACCTAACCAACATCTA

1651 GTGAGGCGATCGGATCCGAGCCTAAACAAATCATTCTTACCCCTCTCCA  
CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCAAACCAAATATGGTTACCATTATTTGATAGACAGATTACT  
ACCACTGGGTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTCTGCCATGAAAATAGATGGCTAAATAAAAAA  
GAGAACGTACACACACAGGACGGTACTTTATCTACCGAATTATTTT

1801 GGACATTGTAATTTGTAAGTGAACTGAAAGGACAGCAAGTTATATCGAATTCC  
CCTGTAACATTAAAACATTGACTTCCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG  
ACGTC

Total number of bases is: 1855.

Sequence name: PAP272

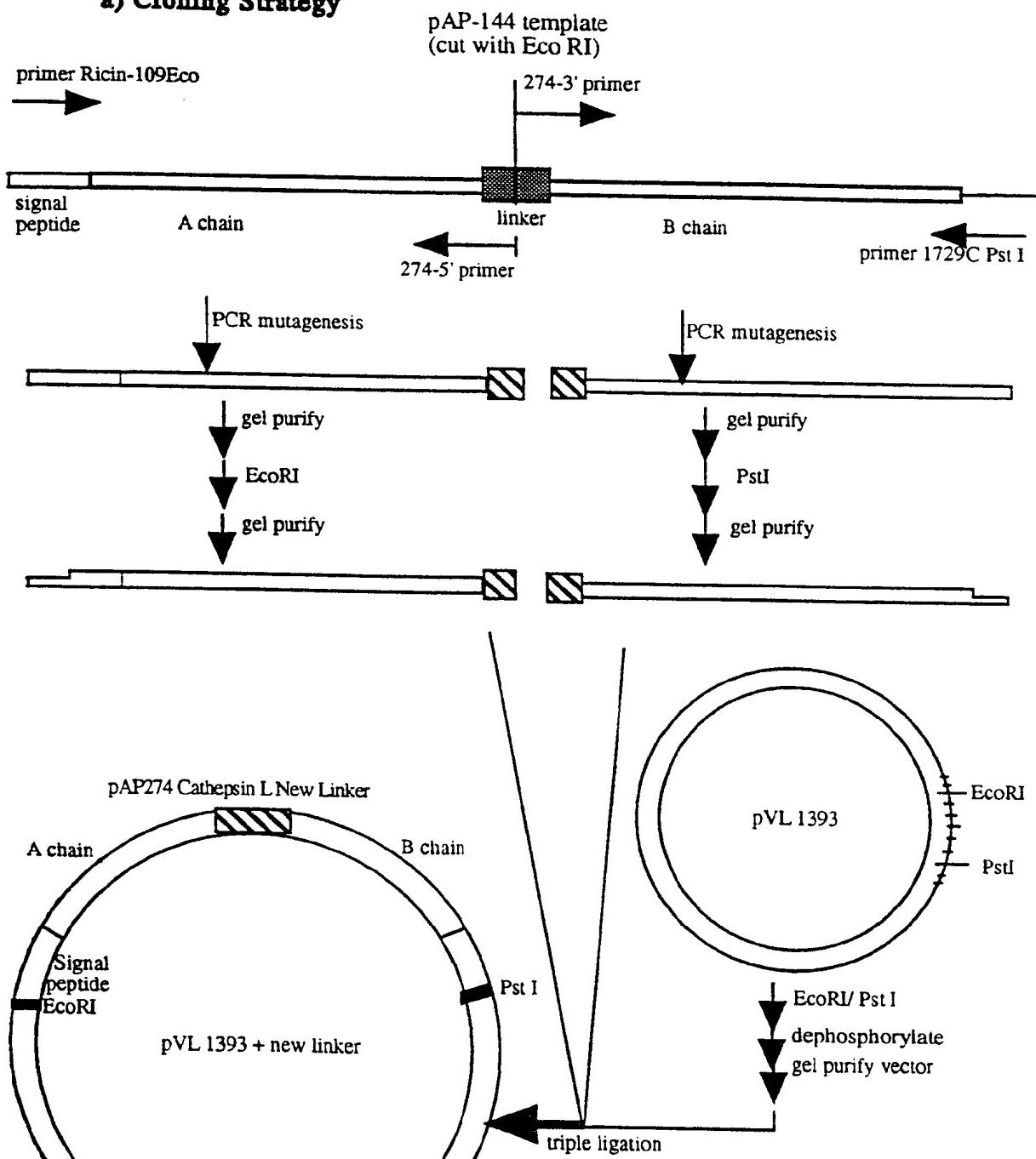
162/254

**FIGURE 35D**

**Amino acid sequence Comparison of Mutant Preproricin Linker  
region of Cathepsin B Site 2 to Wild Type**

Wild type ricin linker: A chain- S L L I R P V V P N F N -B chain  
pAP-272 (Cathepsin B 2)linker: A chain- S L L I A R R M P N F N -B chain

163/254

**FIGURE 36A****PCR Mutagenesis of Prepricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

164/254

**FIGURE 36B****Sequence of Cathepsin L Linker Region****WT preprocin linker**

primer 274-3'  
5' - TCATGGGCTAATTTAATGCTGATGTTGT - 3'  
| \*\*\*\*\* \*  
----- TCTTGCTTATAAGGCC | GTGGTACCAAATTTAAT -----  
----- AGAACGAATATTCCGGT | CACCATGGTTAAAATTA -----  
\*\*\* \*  
3' - AGCAGTGTCAAAAGAAACGAATATAAGGCC - 5'  
primer 274-5'

1) PCR mutagenesis

2) Ligate with pVL1393

**pAP 274 linker  
(Cathepsin L variant)**

----- TCTTGCTTATAATTCCGG | TCATGGGCTAATTTAAT -----  
----- AGAACGAATATAAGGCC | AGTACCCGATTAAAATTA -----

165/254

**FIGURE 36C (P1)**

Sequence of pAP274 insert

1	10   GAATT	20   ACTGAA	30   ACCGGGAG	40   GAAATAC	50   TATTGT
---	------------------	-------------------	---------------------	--------------------	-------------------

1 GAATTCACTGAAACCGGGAGGAAATACTATTGTAAATATGGATGTATGCAGT  
CTTAAGTACTTTGCCCTCCTTATGATAACATTATACCTACATACGTCA

51 GGCAACATGGCTTGTTGGATCCACCTCAGGGTGGTCTTCACATTAG  
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC

101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAAGTTTACCA  
TCCTATTGTTGTATAAGGGTTGTTATGGGTTAATATTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTTCGCGG  
CGCCCACGGTGACACGTTGATGTGTTGAAATAGTCTCGACAAGCGCC

201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTTGCCAA  
AGCAAATTGTTGACCTCGACTACACTCTGTACTATGGTCACAACGGTT

251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACCTCTCA  
TGTCTAACCAACGGATATTGGTTGCCAAATAAACCTGAGAGT

301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA  
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTCTTCATCCTGACA  
ACACCAGCCGATGGCACGACCTTATCGGTATAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTCACTGATGTTAAAAT  
TAGTCCTTCTACGTCTCGTTAGTGAGTAGAAAGTGAACACTAACAGTTTA

451 CGATATACTCGCCTTGGTGGTAATTATGATAGACTTGAACAACTTGC  
GCTATATGTAAGCGGAAACCACCATTAATAACTATCTGAACCTGTTGAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG  
ACCATTAGACTCTCTTATAGCTAACCCCTTACCACTGATCTCCTCC

551 CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCAA  
GATAGAGTCGCGAAATAATAATGTCATGACCAACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTCTTATAATTGCAATCCAATGATTTCAGAACAGCAAG  
GACCGAGCAAGGAAATATTAAACGTAGTTACTAAAGTCTCGTGTTC

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA  
TAAGGTTATATAACTCCCTTTACCGTGCTTTAATCCATGTTGGCCT

166/254

**FIGURE 36C (P2)**

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA  
 CTAGACGTGGTAGGATCGCATTAATGTGAACCTTATCAACCCCCCTCT  
  
 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT  
 GAAAGGTGACGTTAACGTTCTCAGATTGGTCCTCGGAAACGATCAGGTTA  
  
 801 TCAAATGCAAAGACGTAATGGTCCAAATTCAAGTGTACGATGTGAGTA  
 AGTTGACGTTCTGCATTACCAAGGTTAACGTCACACATGCTACACTCAT  
  
 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA  
 ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACGCGTGGAGGTGGT  
  
 901 TCGTCACAGTTCTTGCTTATATTCCGGTACATGGCTAATTAAATGC  
 AGCAGTGTCAAAAGAAAGGAATATAAGGCCAGTACCCGATTAAAATTACG  
  
 951 TGATGTTGTATGGATCCTGAGCCATAGTGCCTATCGTAGGTCGAAATG  
 ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
 CAGATACACAACATAATCCCTACCTTCTAACGTTGCTGCCTTGCCTTAT  
  
 1051 CAGTTGTGCCATGCAAGTCTAACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTAGATTATGTCTACGTTAGTCGAGACCTGAAA  
  
 1101 GAAAAGAGACAATACTATTGATCTAACGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
  
 1151 GGTACAGTCCGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA  
 CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT  
  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC  
 TGACTACGGTGGCGACCGTTATACCTTACCTTGGTAGTATTAGG  
  
 1251 CAGATCTAGTCTAGTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
 GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTCACCATGGTGTG  
  
 1301 TTACAGTGCAAACCAACATTATGCCGTTAGTCAGGTTGGCTTCTACT  
 AATGTCACGTTGGTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA  
  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
 TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC  
  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
 GAACGTTCGTTATCACCTGTTACACCTATCTCCTGACATCGTCACCTT

167/254

**FIGURE 36C (P3)**

1451 AGGCTGAACAAACAGTGGGCTCTTATGCAGATGGTCATAACGTCTCAG  
TCCGACTTGTGTCACCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATAACGGAAACAGT  
GTTTGCGCTCTATTAACGGAATGTTCACTAAGATTATGCCCTTGTCA

1551 TGTAAAGATCCTCTCTGTGGCCCTGCATCCTCTGCCAACGATGGATGT  
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTTAAATTGTATAGTGGATTGGTAGAT  
AGTTCTTACTACCTTGGTAAATTTAACATATCACCTAACCAACATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTACCCCTCTCCA  
CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCAAACCAAATATGGTTACCATTTATTTGATAGACAGATTACT  
ACCACTGGGTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA

1751 CTCTGCAGTGTGTGTCTGCCATGAAAATAGATGGCTTAAATAAAAAA  
GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATTTT

1801 GGACATTGAAATTGTAACTGAAAGGACAGCAAGTTATCGAATTCC  
CCTGTAACATTTAAACATTGACTTCCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG  
ACGTC

Total number of bases is: 1855.

Sequence name: PAP274

168/254

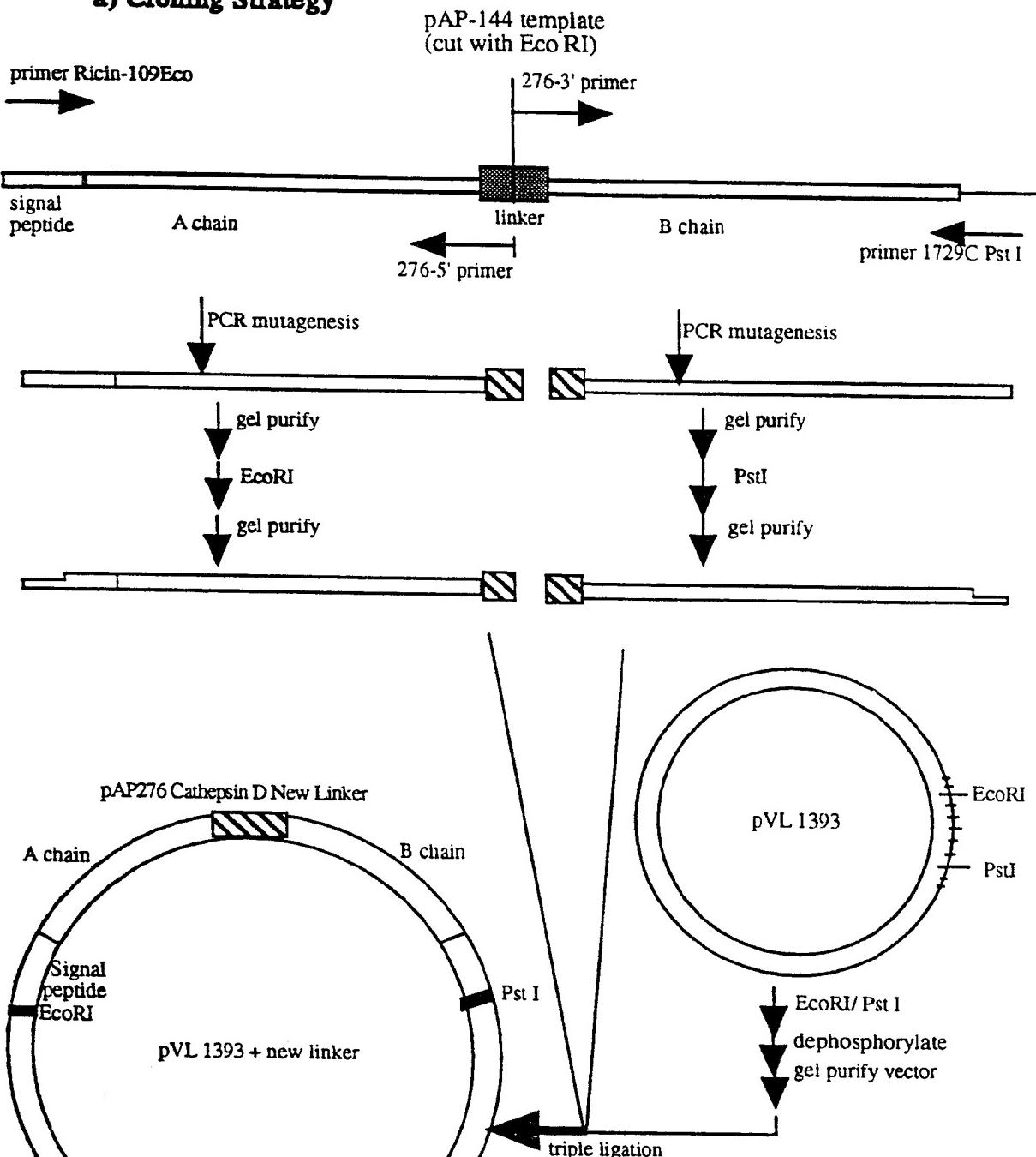
**FIGURE 36D**

**Amino acid sequence Comparison of Mutant Preproricin Linker  
region of Cathepsin L to Wild Type**

Wild type ricin linker:            A chain- S L L I R P V V P N F N -B chain

pAP-274 (Cathepsin L) linker: A chain- S L L I F R S W A N F N -B chain

169/254

**FIGURE 37A****PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

170/254

**FIGURE** 37B

## **Sequence of Cathepsin D Linker Region**

### WT preprocin linker

primer 276-3'

5' - ACTGTTATTGTTATCACCGCTGATGTTGT - 3'

| \* \* \* \* \* \* \* \* \* \* \* \*

----- TCTTGCTTATAAGGCCA | GTGGTACCAAATTTAAT -----

----- AGATAACGAATATTCCGG | CACCATGGTTAAAATTA -----

\* \* \* \* \* \* \* \* \* \* \* \*

3' - AGCAGTGTCAAAAGACCACAAACAGTAGCGA - 5'

primer 276-5'

- 1) PCR mutagenesis
  - 2) Ligate with pVL1393

### pAP 276 linker

#### **(Cathepsin D variant)**

- - - TCTGGTGTGTCATCGCT | ACTGTTATTGTTATCACC - - -  
- - - AGACCACAAACAGTAGCGA | TGACAATAACAAATAGTGG - - -

171/254

**FIGURE 37C (P1)**

Sequence of pAP276 insert

10	20	30	40	50

1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT  
 CTTAAGTACTTGCCCTCTTATGATAACATTACCTACATACGTCA  
 51 GGCAACATGGTTGTTGGATCCACCTCAGGGTGGTCTTCACATTAG  
 CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC  
 101 AGGATAACAACATATTCCCCAACAAACATACCCAATTATAAACTTTACCA  
 TCCTATTGTTGTATAAGGGTTGTTATGGGTTAATATTTGAAATGGTGT  
 151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTTCGCGG  
 CGCCCACGGTGACACGTTCGATGTGTTGAAATAGTCTCGACAAGCGCC  
 201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGGTGC  
 AGCAAATTGTTGACCTCGACTACACTCTGTACTATGGTCACAACGGTT  
 251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACCTCA  
 TGTCTAACCAACGGATATTGGTTGCCAAATAAAATCAACTTGAGAGT  
 301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA  
 TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT  
 351 TGTGGTCGGCTACCGTGGAAATAGCGCATATTTCTTCATCCTGACA  
 ACACCAGCCGATGGCACGACCTTATCGCGTATAAAGAAAGTAGGACTGT  
 401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTCACTGATGTTAAAAT  
 TAGTCCTCTACGTCTCGTTAGTGAGTAGAAAGTGAACAGTTA  
 451 CGATATACTCGCCTTGGTGGTAATTATGATAGACTTGAACAAACTTGC  
 GCTATATGTAAGCGGAAACCACCATTAATAACTATCTGAACCTGAAACG  
 501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG  
 ACCATTAGACTCTCTTATAGCTAACCCCTTACCAAGGTGATCTCCTCC  
 551 CTATCTAGCGCTTATTACAGTACTGGTGGCACTCAGCTTCCA  
 ACTGATAGAGTCGCGAAATAATAATGTCATGACCAACCGTGAGTCGAAGGTTGA  
 601 CTGGCTCGTTCTTATAATTGCATCCAAATGATTCAAGCAGCAAG  
 GACCGAGCAAGGAAATATTAACGTAGGTTACTAAAGTCTCGTCGTT  
 651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA  
 TAAGGTTATATAACTCCCTCTTACCGCGTCTTAATCCATGTTGGCCT

172/254

**FIGURE 37C (P2)**

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA  
 CTAGACGTGGCTAGGATCGCATTAAATGTGAACCTTATCAACCCCTCT  
  
 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT  
 GAAAGGTGACGTTAAGTTCTCAGATTGGTCCTCGGAAACGATCAGGTTA  
  
 801 TCAACTGCAAAGACGTAATGGTCAAATTCACTGTGTACGATGTGAGTA  
 AGTTGACGTTCTGCATTACCAAGGTTAACGTCACACATGCTACACTCAT  
  
 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA  
 ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACGCGTGGAGGTGGT  
  
 901 TCGTCACAGTTCTGGTGTTCATCGCTACTGTTATTGTTATCACCGC  
 AGCAGTGTCAAAAGACCACAACAGTAGCGATGACAATAACAATAGTGGCG  
  
 951 TGATGTTGTATGGATCCTGAGCCCATACTGCGTATCGTAGGTCGAAATG  
 ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAAACGGAAACGCAATA  
 CAGATAACACAACATACTACCTACCTCTAAGGTGTTGCCTTGCCTT  
  
 1051 CAGTTGTGCCATGCAAGTCTAATAACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTCAAGATTATGTCTACGTTAGTCGAGACCTGAAA  
  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAACTGCTGCA  
 CCATGTCAGGCCCTCAGATAACACTAGATACTAACGTTATGACGACGT  
  
 1201 ACTGATGCCACCCGCTGGCAAATATGGATAATGGAACCATCATAAATCC  
 TGACTACGGTGGCGACCGTTATACCCATTACCTGGTAGTATTAGG  
  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
 GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTGTCACCATGGTGTG  
  
 1301 TTACAGTGCAAACCAACATTATGCCGTTAGTCAAGGTTGGCTTCTACT  
 AATGTCACGTTGGTTGTAACACGGCAATCAGTCCAACCGAAGGATGA  
  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
 TTATTATGTGTTGGAAAACAATGTTGTAACAACCCGATATAACCAGACAC  
  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
 GAACGTTCGTTATCACCTGTTACACCTATCTCCTGACATCGTCACTTT

173/254

**FIGURE 37C (P3)**

1451 AGGCTGAACAAACAGTGGCTTTATGCAGATGGTCAATACGTCCAG  
TCCGACTTGTGTCACCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGAAACAGT  
GTTTGGCTCTATTACCGAATGTTCACTAAGATTATGCCCTTGTCA

1551 TGTAAAGATCCTCTCTGTGGCCCTGCATCCTCTGCCAACGATGGATGT  
ACAATTCTAGGAGAGAACACCGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTAAATTGTATAGTGGATTGGTGTAGAT  
AGTTCTTACTACCTTGGTAAATTTAAACATATCACCTAACCAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTAAACAAATCATTCTTACCCCTCCA  
CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCAAACCAAATATGGTTACCATTATTTGATAGACAGATTACT  
ACCACTGGGTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGCCTGCCATGAAAATAGATGGCTAAATAAAAAA  
GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATTTT

1801 GGACATTGTAATTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC  
CCTGTAACATTAAAACATTGACTTCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG  
ACGTC

Total number of bases is: 1855.

Sequence name: PAP276

174/254

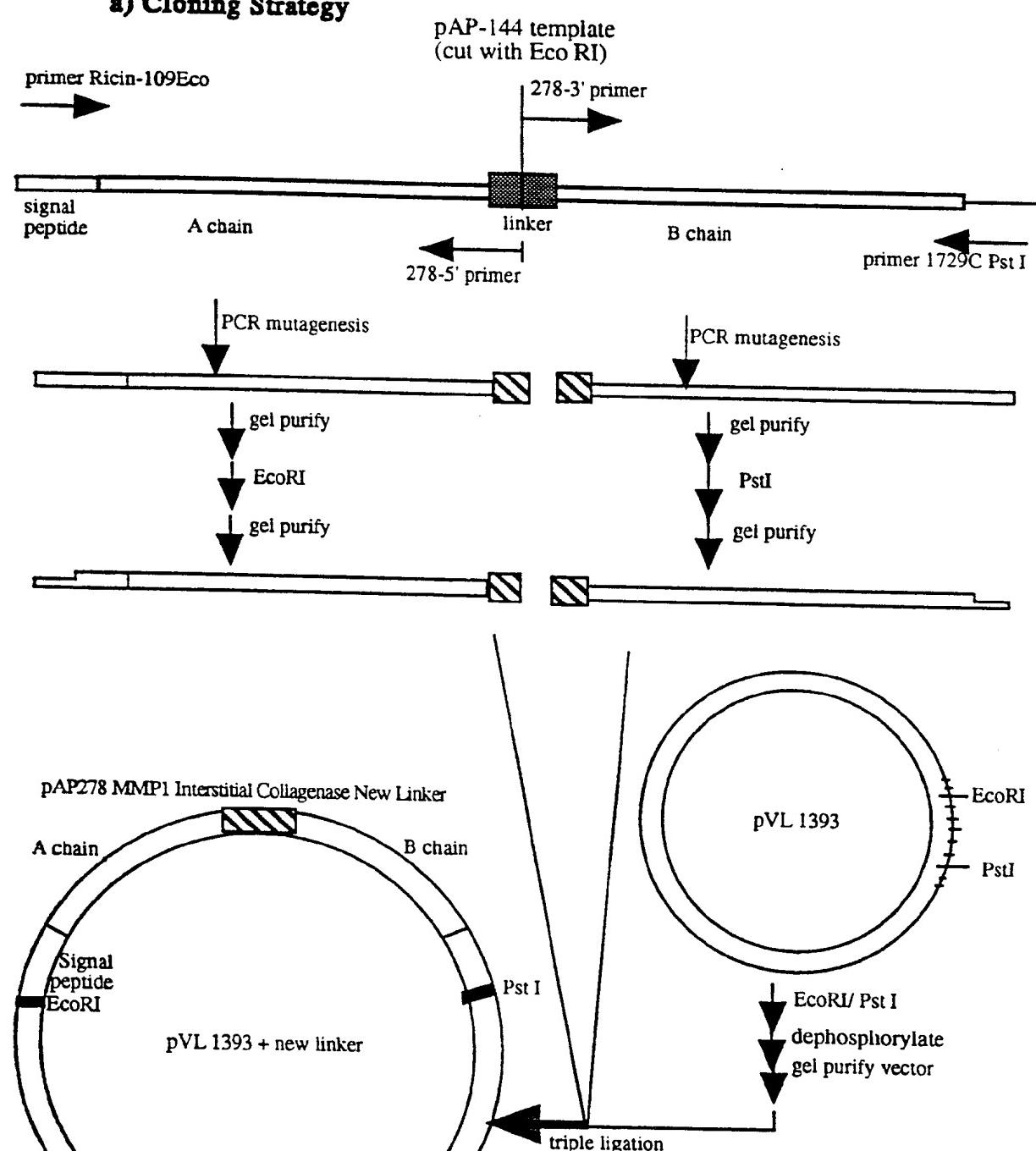
**FIGURE 37D**

**Amino acid sequence Comparison of Mutant Preproricin Linker  
region of Cathepsin D to Wild Type**

Wild type ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-276 (Cathepsin D) linker: A chain- S G V V I A T V I V I T -B chain

175/254

**FIGURE 38A****PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

176/254

**FIGURE 38B****Sequence of MMP-1 (Interstitial collagenase) Linker Region****WT preprocin linker**

primer 278-3'  
5' - ATTTGGGGACAGTTAATGCTGATGTTGT - 3'  
\* \* \* \* \* \* \* \*  
----- TCTTGCTTATAAGGCCA | GTGGTACCAAATTTAAT -----  
----- AGAAACGAATATTCCGGT | CACCATGGTTAAAATTA -----  
\* \* \* \* \* \* \* \*  
3' - AGCAGTGTCAAAAGAAACCCAGGAGTTCCG - 5'  
primer 278-5'

1) PCR mutagenesis

2) Ligate with pVL1393

**pAP 278 linker  
(MMP-1 variant)**

----- TCTTGGGTCCCTCAAGGC | ATTTGGGGACAGTTAAT -----  
----- AGAAACCCAGGAGTTCCG | TAAACCCCTGTCAAATTA -----

177/254

**FIGURE 38C (P1)**

Sequence of pAP278 insert

1	10 	51	20 	101	30 	151	40 	201	50 
---	--------	----	--------	-----	--------	-----	--------	-----	--------

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1  GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT
   CTTAAGTACTTGGCCCTCCTTATGATAACATTATACTACATACGTCA

51  GGCAACATGGCTTGTTGGATCCACCTCAGGGTGGCTTCACATTAG
   CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC

101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
   TCCTATTGTTGTATAAGGGTTGTTATGGGTTAATATTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTTCGCGG
   CGCCCACGGTACACGTTCGATGTGTTGAAATAGTCTCGACAAGCGCC

201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATAACCAGTGGTGC
   AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAAC
   TGTCACCAACAAACGGATATTGGTTGCCAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
   TTAGTACGTCTCGAAAGACAATGTAATCGCACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGCTGAAATAGCGCATATTCTTCATCCTGACA
   ACACCAAGCCGATGGCACGACCTTATCGGTATAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTCACTGATGTTCAA
   TAGTCCTTACGTCTCGTTAGTGAGTAGAAAGTGA
   CTACAAGTTA

451 CGATATACATTGGCTTGTTGGTAATTATGATAGACTTGAA
   GACAACATTGC
   GCTATATGTAAGCGAAACCACCATTAATACTATCTGAAC
   TTGTTGAACG

501 TGGTAATCTGAGAGAAAATATGAGTTGGAAATGGTCCACTAGAGGAGG
   ACCATTAGACTCTTTAGCTAACCCCTTACCAAGGTGATCTCCTCC

551 CTATCTAGCGCTTATTACAGTACTGGTGGCACTCAGCTTCAACT
   GATAGAGTCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTTCTTATAATTGCATCCAAATGATTCAGAAC
   GAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGT
   CGTTC

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA
   TAAGGTTATATAACTCCCTTTACCGTGCTTTAATCCATGTTGGCCT

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178/254

**FIGURE 38C (P2)**

701 GATCTGACCCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA  
CTAGACGTGGTCTAGGATCGCATTAATGTGAACCTTATCAACCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT  
GAAAGGTGACGTTAAGTTCTCAGATTGGTCCTCGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTCAAATTCAAGTGTACGATGTGAGTA  
AGTTGACGTTCTGCATTACCAAGGTTAACGTACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTATGGGTATAGATGCCACCTCCACCA  
ATAATTAGGGATAGTATCGAGAGTACCACATATCACGCGTGGAGGTGGT

901 TCGTCACAGTTCTTGCGGTCCTCAAGGCATTGGGACAGTTAATGC  
AGCAGTGTAAAAGAAACGCAGGAGTCCGTAACACCTGTCAAATTACG

951 TGATGTTGTATGGATCCTGAGCCCAGTGCCTAGTCGTAGGTGAAATG  
ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC

1001 GTCTATGTGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
CAGATACACAACATACTACATCCCTACCTCTAACGGTGTGCCTTGCCTAT

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
GTCAACACCGGTACGTTAGATTATGTCTACGTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTACG  
CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA  
CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC  
TGACTACGGTGGCGACCGTTATACCTTACCTTGGTAGTATTAGG

1251 CAGATCTAGTCTAGTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTCACCATGGTGTG

1301 TTACAGTGCAAACCAACATTATGCCGTTAGTCAAGGTTGGCTTCTACT  
AATGTCAGTTGGTTGTAACACGGCAATCAGTTCAACCGAAGGATGA

1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
GAACGTTCGTTATCACCTGTTACACCTATCTCCTGACATCGTCACCTT

179/254

**FIGURE 38C (P3)**

1451 AGGCTGAACAAACAGTGGCTCTTATGCAGATGGTTCAATAACGTCTCAG  
TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATAACGGAAACAGT  
GTTTGGCTCTATTAACGGAATGTTCACTAAGATTATGCCCTTGTCA

1551 TGTAAAGATCCTCTTGTGCCCTGCATCCTCTGGCCAACGATGGATGT  
ACAATTCTAGGAGAGAACACCGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTAAATTGTATAGTGGATTGGTAGAT  
AGTTCTTACTACCTGGTAAAATTAAACATATCACCTAACCAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTAAACAAATCATTCTTACCCCTCTCCA  
CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGAGAGGT

1701 TGGTGAACCAAACAAATATGGTTACCATTATTTGATAGACAGATTACT  
ACCACTGGTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTGCCATGAAAATAGATGGCTAAATAAAAAA  
GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATTTT

1801 GGACATTGTAATTTGTAAGTGAAAGGACAGCAAGTTATATCGAATTCC  
CCTGTAACATTTAAACATTGACTTCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG  
ACGTC

Total number of bases is: 1855.

Sequence name: PAP278

180/254

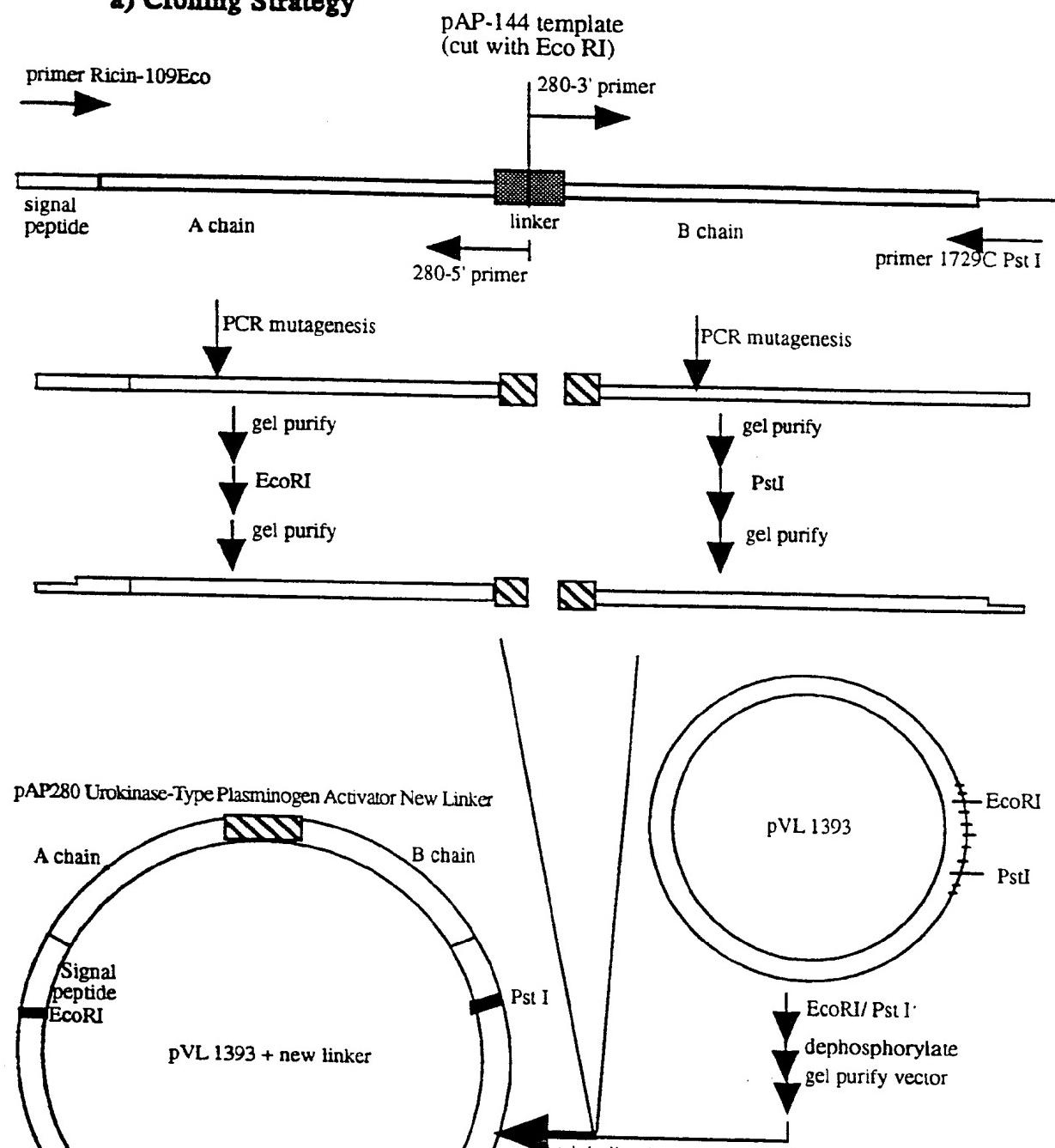
**FIGURE 38D**

**Figure 38. d) Amino acid sequence Comparison of Mutant Preproricin Linker region of MMP-1 (Interstitial collagenase) to Wild Type**

Wild type ricin linker:                   A chain- S L L I R P V V P N F N -B chain

pAP-278 (MMP-1) linker:               A chain- S L G P Q G I W G Q F N -B chain

181/254

**FIGURE 39A****PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

182/254

**FIGURE 39B****Sequence of Urokinase-Type Plasminogen Activator Linker Region****WT preprocin linker**

primer 280-3'

5' - GTTGTGGTGGCTCTGTAGCTGATGTTGT - 3'  
\* \* \* \* \* \* \* \* \*

```

----- TCTTGCTTATAAGGCCA | GTGGTACCAAATTAAAT -----
----- AGAAACGAATATTCCGGT | CACCATGGTTAAAATTA -----
* * * * * * * * * * * *
3' - AGCAGTGTCAAATTTTAGGGGACCTTCT - 5'
      primer 280-5'

```

1) PCR mutagenesis

2) Ligate with pVL1393

**pAP 280 linker****(uPA variant)**

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----- AAAAAATCCCCTGGAAGA | GTTGTGGTGGCTCTGTA -----
----- TTTTTAGGGGACCTTCT | CAACAGCCACCGAGACAT -----

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183/254

**FIGURE 39C (P1)**

Sequence of pAP280 insert

10	20	30	40	50

1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT  
 CTTAAGTACTTGGCCCTCCTTATGATAACATTACACATACAGTC  
 51 GGCAACATGGTTGTTGGATCCACCTCAGGGTGGTCTTCACATTAG  
 CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAA  
 101 AGGATAACAACATATTCCCCAAACAATACCCAATTATAAACTTACCA  
 TCCTATTGTTGTATAAGGGTTGTTATGGGTTAATATTGAAATGGTGT  
 151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTTGCC  
 CGCCCACGGTGACACGTTCGATGTGTTGAAATAGTCTCGACAAGCGCC  
 201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATAACAGTGGTGC  
 AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGT  
 251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACCTCA  
 TGTCTAACCAAACGGATATTGGTTGCCAATAAAATCAACTTGAGAGT  
 301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA  
 TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT  
 351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTCTTCATCCTGACA  
 ACACCAGCCGATGGCACGACCTTATCGCGTATAAAGAAAGTAGGACTGT  
 401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTCACTGATGTTAAAAT  
 TAGTCCTCTACGTCTCGTTAGTGAGTAGAAAAGTGA  
 451 CGATA  
 501 TGGTAATCTGAGAGAAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG  
 ACCATTAGACTCTCTTTATAGCTCAACCCTTACCAAGGTGATCTCCTCC  
 551 CTATCTAGCGCTTATTACAGTACTGGTGGCACTCAGCTTCAACT  
 GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA  
 601 CTGGCTCGTTCTTATAATTGCATCCAAATGATTCAGAACAGCAAG  
 GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTCGTT  
 651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA  
 TAAAGGTTATATAACTCCCTTTACCGTGCTTTAATCCATGTTGGCCT

184/254

**FIGURE 39C (P2)**

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA  
 CTAGACGTGGTCTAGGATCGCATTAATGTGAACCTTATCAACCCCCCTCT  
 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT  
 GAAAGGTGACGTTAAGTTCTCAGATTGGTCCTCGGAAACGATCAGGTTA  
 801 TCAACTGCAAAGACGTAATGGTCCAAATTCAAGTGTACGATGTGAGTA  
 AGTTGACGTTCTGCATTACCAAGGTTAACGTCACACATGCTACACTCAT  
 851 TATTAATCCTATCATAGCTCTCATGGTGTAGATGCCACCTCCACCA  
 ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACGCGTGGAGGTGGT  
 901 TCGTCACAGTTAAAAAATCCCCTGGAAGAGTTGTCGGTGGCTCTGTAGC  
 AGCAGTGTCAAATTAGGGACCTCTCAACAGCCACCGAGACATCG  
 951 TGATGTTGTATGGATCCTGAGCCCAGTGCCTACGTAGGTCGAAATG  
 ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGAGATTCCACAACGGAAACGCAATA  
 CAGATACACAACATACTACCCCTACCTCTAACGGTTAGTCGCTTGCCTTGC  
 1051 CAGTTGTGCCATGCAAGTCTAACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTAGATTATGTCAGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAACGGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCGGGAGTCTATGTGATGATCTATGATTGCAAACTGCTGCA  
 CCATGTCAGGCCCTCAGATAACACTACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCGCTGGCAAATATGGATAATGGAACCATCATAAATCC  
 TGACTACGGTGGGCGACCGTTAACCCCTATTACCTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTAGCAGCGACATCAGGGAACAGTGGTACCAAC  
 GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTCACCATGGTGTG  
 1301 TTACAGTGCACCAACACATTATGCCGTTAGTCAAGGTTGGCTTCTACT  
 AATGTCACGTTGGTTGTAACACGGCAATCAGTTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
 TTATTATGTGTTGGAAAACAATGTTGGTAACAAACCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
 GAACGTTCGTTATCACCTGTTACACCTATCTCCTGACATCGTCACCTT

185/254

**FIGURE 39C (P3)**

1451 AGGCTGAACAACAGTGGCTTTATGCAGATGGTCAATAACGTCTCAG  
TCGGACTTGTGTCACCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAACCGAGATAATTGCCTTACAAGTGATTCTAATATAACGGAAACAGT  
GTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTGTCA

1551 TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTAAATTGTATAGTGGATTGGTGTAGAT  
AGTTCTTACTACCTTGGTAAAATTAAACATATCACCTAACCAACATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTACCCCTCTCCA  
CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCAAACAAATATGGTTACCATTATTTGATAGACAGATTACT  
ACCACTGGGTTGGTTATACCAATGTAATAAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTCTGCCATGAAAATAGATGGCTTAAATAAAAAA  
GAGAACGTACACACACAGGACGGTACCTTATCTACCGAATTATTTT

1801 GGACATTGTAATTTGTAAGTGAAGGACAGCAAGTTATATCGAATTCC  
CCTGTAACATTAAACATTGACTTCCGTGTTCAATATAGCTTAAGG

1851 TGCAG  
ACGTC

Total number of bases is: 1855.

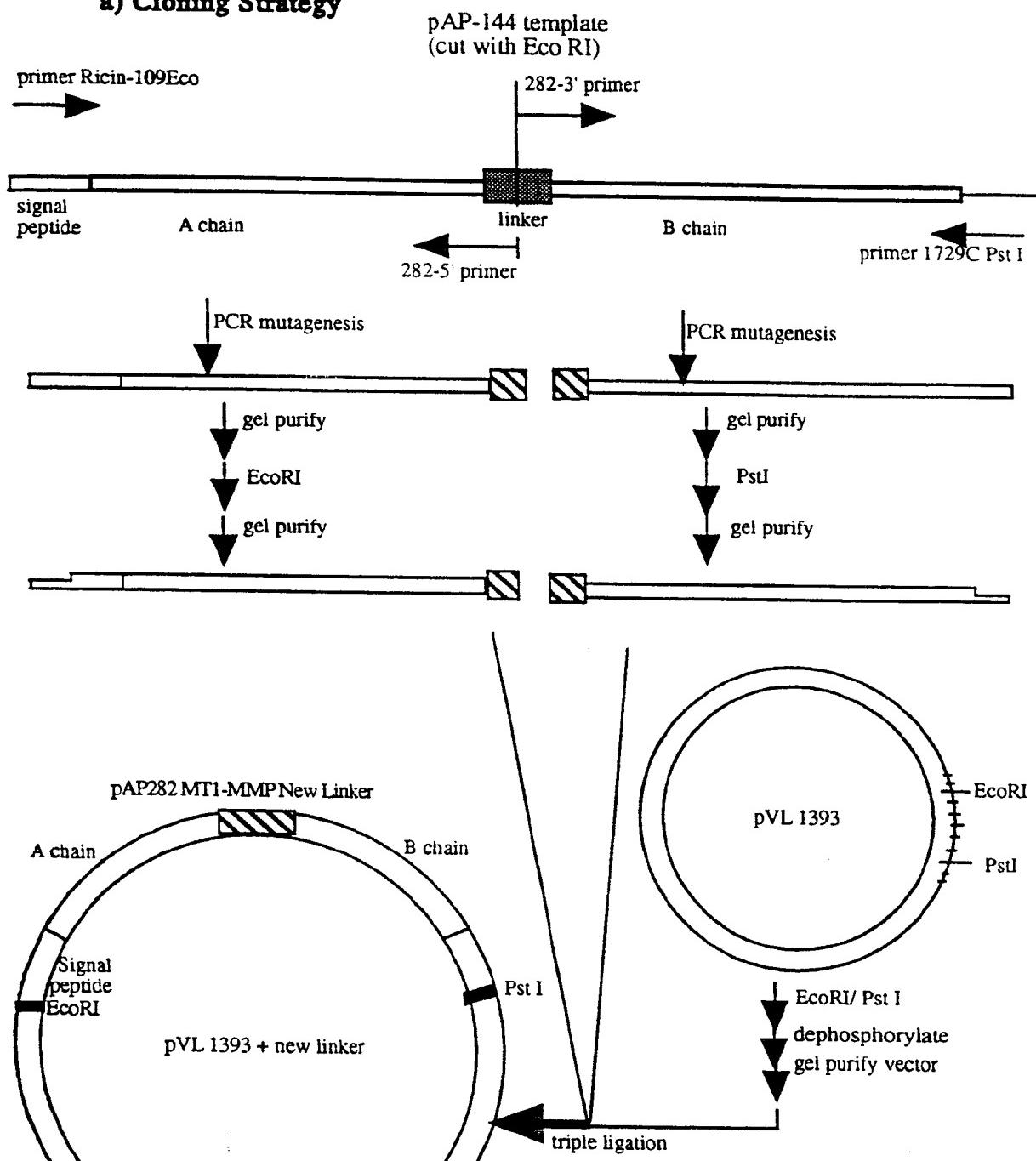
Sequence name: PAP280

186/254

**FIGURE 39D****Figure 39. d) Amino acid sequence Comparison of Mutant Preproricin Linker region of Urokinase-Type Plasminogen Activator to Wild Type**

Wild type ricin linker:                   A chain- S L L I R P V V P N F N -B chain  
pAP-280 (uPA) linker:                   A chain- K K S P G R V V G G S V-B chain

187/254

**FIGURE 40A****PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

188/254

## FIGURE 40B

## **Sequence of MT-MMP Linker Region**

### WT preprocin linker

**primer 282-3**

5' - GCTCCTGGTATTGTTGGGGGTG - 3'

\*\*\*\*\* \* \* \* \*

- TCTTTGCTTATAACCCCA | GCGGGTTC -

-AGAAAACGAATAATTCCGGT|GAGGCTTGTATTAAT-----

\* \* \* \* \* \* \* \* \* \* \* \* \* \* \* | C A C C A T G G T T T A A A A T T A - - - - - - - - -

AGGGGTTCTGAGGATCCC 51

**Primer 282-5'**

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12 ECB - European Central Bank | Page

#### 1) PCR mutagenesis

### 2) Ligate with pVT.1393

(MT-MMP var)

-CTAGGG | GCTCCTG

-CCCCAAGGACTCCTAGGG| GCTCCTGGTATTCTTGGC -

-GGGGTTCCCTGAGGATCCC|CGAGGACCATAAGAACCG-

MANUFACTURED -

**FIGURE 40C (P1)**

Sequence of pAP282 insert

1	10 	20 	30 	40 	50 
---	--------	--------	--------	--------	--------

GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT  
 CTTAAGTACTTGGCCCTCCTTATGATAACATTACCTACATACGTCA

51 GGCAACATGGCTTGTTGGATCCACCTCAGGGTGGTCTTCACATTAG  
 CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC

101 AGGATAACAACATATTCCCCAACAAATACCCAATTATAAAGTTACCA  
 TCCTATTGTTGTATAAGGGTTGTTATGGTTAATATTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTTCGCGG  
 CGCCCACGGTGACACGTTGATGTGTTGAAATAGTCTCGACAAGCGCC

201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGGTCCAA  
 AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACCTCTCA  
 TGTCTCAACCAAACGGATATTGGTTGCCAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA  
 TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGGAAATAGCGCATATTCTTCATCCTGACA  
 ACACCAGCCGATGGCACGACCTTATCGCGTATAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAACATCACTCATCTTCACTGATGTTAAAAT  
 TAGTCCTCTACGTCTCGTTAGTAGAAAGTAGACTACAAGTTTA

451 CGATATACATTGCCTTGGTGGTAATTATGATAGACTTGAACAACTTGC  
 GCTATAATGTAAGCGGAAACCACCATTAATAACTATCTGAACCTGTTGAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG  
 ACCATTAGACTCTTTATAGCTCAACCCTTACCAAGGTGATCTCCTCC

551 CTATCTAGCGCTTATTATTACAGTACTGGTGGCACTCAGCTTCAA  
 ACTGATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTTCTTATAATTGCATCCAAATGATTCAGAACAGCAAG  
 GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTCGTT

651 ATTCCAATATAATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA  
 TAAGGTTATATAACTCCCTCTTACCGCGTCTTAATCCATGTTGGCCT

190/254

**FIGURE 40C (P2)**

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAAGTTGGGGAGA  
 CTAGACGTGGTCTAGGATCGCATTAATGTGAACCTTATCAACCCCTCT  
 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT  
 GAAAGGTGACGTTAACGGTCTCAGATTGGTCTCGGAAACGATCAGGTTA  
 801 TCAACTGCAAAGACGTAATGGTCCAAATTCAAGTGTACGATGTGAGTA  
 AGTTGACGTTCTGCATTACCAAGGTTAACGTACACACATGCTACACTCAT  
 851 TATTAATCCCTATCATAGCTCTCATGGGTATAGATGCGCACCTCCACCA  
 ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACGCGTGGAGGTGGT  
 901 TCGTCACAGTTCCCCAAGGACTCCTAGGGCTCCTGGTATTCTGGCGC  
 AGCAGTGTCAAAGGGTCTGAGGATCCCCGAGGACCATAAGAACCGCG  
 951 TGATGTTGTATGGATCCTGAGCCCATACTGCGTATCGTAGGTCGAAATG  
 ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
 CAGATACACAACATACTACCTACCTTCTAAGGTGTTGCCCTTGCCTTAC  
 1051 CAGTTGTGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
 GTCAACACCGGTACGTTAGATTGTCTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
 CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA  
 CCATGTCAGGCCCTCAGATAACACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGATAATGGAACCACATCAAATCC  
 TGACTACGGTGGCGACCGTTATACCTTACCTTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTAGCAGCGACATCAGGAAACAGTGGTACCACAC  
 GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTACCATGGTGTG  
 1301 TTACAGTGCAAACCAACATTATGCCGTTAGTCAAGGTTGGCTTCTACT  
 AATGTCACGTTGGTTGAAATACGGCAATCAGTCCAACCGAAGGATGA  
 1351 AATAATAACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
 TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGGACTGTAGCAGTGAA  
 GAACGTTCGTTATCACCTGTTACACCTATCTCCTGACATCGTCACTTT

191/254

**FIGURE 40C (P3)**

1451 AGGCTGAACAAACAGTGGGCTCTTATGCAGATGGTTCAATACGTCTCAG  
TCCGACTTGTGTCACCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGAAACAGT  
GTTTGGCTCTATTAACGGAATGTTCACTAAGATTATGCCCTTGTCA

1551 TGTAAAGATCCTCTCTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAATGATGGAACCATTAAATTGTATAGTGGATTGGTAGAT  
AGTTCTTACTACCTGGTAAAATTAAACATATCACCTAACCAATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTACCCCTCTCCA  
CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT

1701 TGGTACCCAAACCAAATATGGTTACCATTATTTGATAGACAGATTACT  
ACCACTGGTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA

1751 CTCTTGCAGTGTGTGTCTGCCATGAAAATAGATGGCTTAAATAAAAAA  
GAGAACGTACACACACAGGACGGTACTTTATCTACCGAATTATTTT

1801 GGACATTGTAATTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC  
CCTGTAACATTAAAACATTGACTTCCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG  
ACGTC

Total number of bases is: 1855.

Sequence name: PAP282

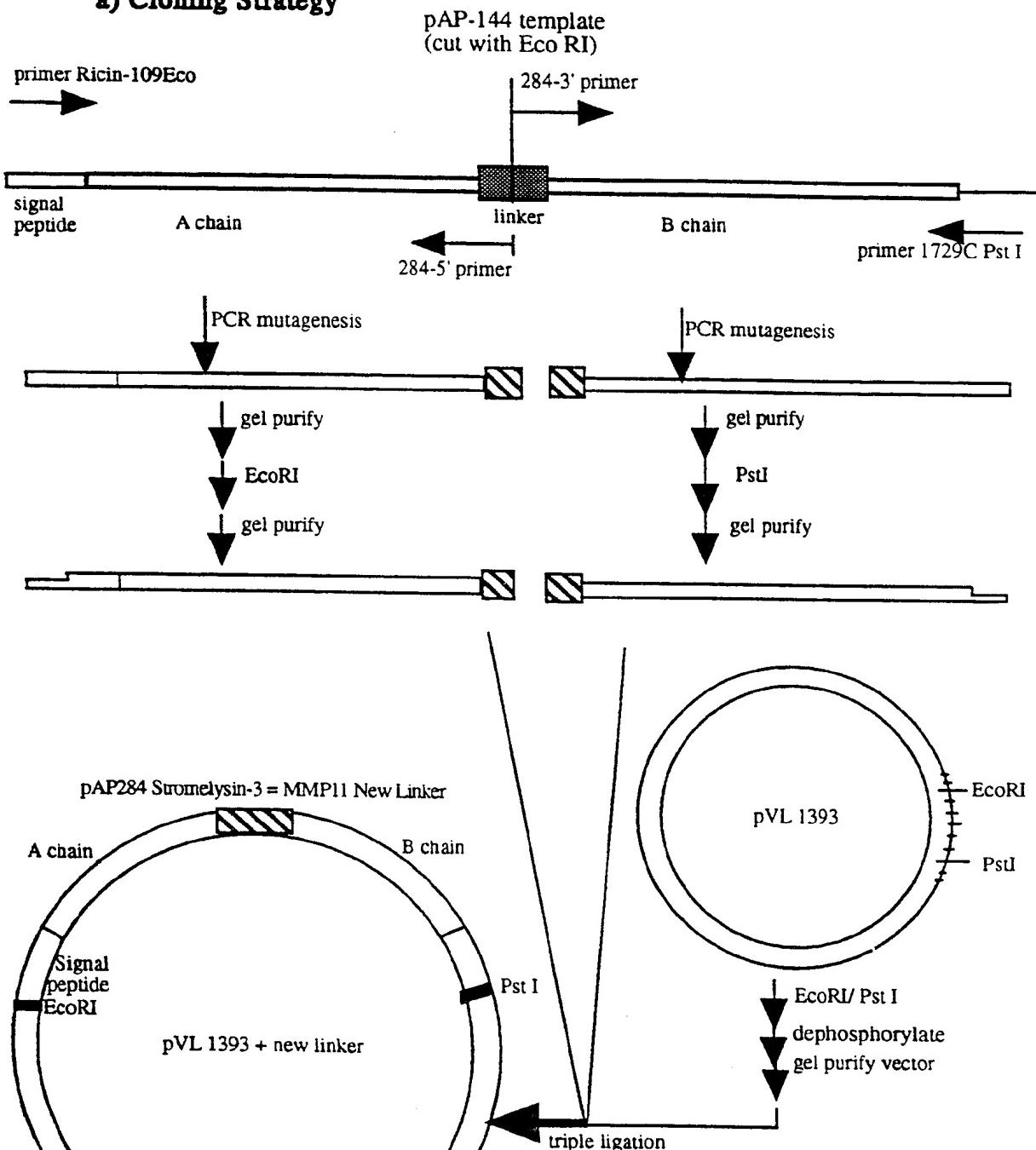
192/254

**FIGURE 40D****Amino acid sequence Comparison of Mutant Preproricin Linker  
region of MT-MMP to Wild Type**

Wild type ricin linker:                   A chain- S L L I R P V V P N F N -B chain

pAP-282 (MT-MMP) linker:               A chain- P Q G L L G A P G I L G-B chain

193/254

**FIGURE 41A****PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

194 / 254

**FIGURE 41B**

**Sequence of MMP-11 (Stromelysin-3) Linker Region**

**WT preprocin linker**

primer 284 - 3'

5' - ATGGAAAGACGCCATGCTCGTTAGTCATGTCGAAGAGCCCTCACACTGCTGATGTTGTATGGAT - 3'

|

----- TCTTTGCTATAAGCCA | GTGGTACCAAATTAAAT -----

----- AGAAACGAATAATTCCGGT | CACCATGGTTAAAATTAA -----

-----

3' - GGTTAGCAGTGTCAAAGTGCCTCCCCAATTCTCACCCCTAAATAACTTAGCTGAGG - 5'

primer 284 - 5'

1) PCR mutagenesis

2) Ligate with pVL1393

**pAP 284 linker**

**(MMP-11 variant)**

----- CACGGCCCGAGGGTTAACAGAGTGGGATTATGAATCTGACGTC | ATGGAAAGGGCCATGCTCGTTAGTCATGTCGAAGAGCCCTCACACT - - -

----- GTGCGGGGGGCTCCCCAATTCTCACCCCTAAATACTTAGACTGAGG | TACCCCTCTCCGGTACGAGCAAATCAAGTACAGCAAATCGGAGTGTGA - - -

195/254

**FIGURE 41C (P1)**

Sequence of pAP284 insert

1	10 	20 	30 	40 	50 
---	--------	--------	--------	--------	--------

GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT  
 CTTAAGTACTTGGCCCTCCTTATGATAACATTATACCTACATAACGTCA  
 GGCAACATGGCTTGTTGGATCCACCTCAGGGTGGTCTTCACATTAG  
 CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC  
 AGGATAACAACATATTCCCCAACAAACAATACCAATTATAAACTTACACAC  
 TCCTATTGTTGATAAGGGTTGTTATGGGTTAATATTGAAATGGTGT  
 GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTTCGCGG  
 CGCCCACGGTGACACGTTGATGTGTTGAAATAGTCTCGACAAGCGCC  
 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTGCCAA  
 AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT  
 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACCTCTCA  
 TGTCTCAACCAAACGGATATTGGTTGCCAATAAAATCAACTTGAGAGT  
 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA  
 TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT  
 TGTGGTCGGCTACCGTGTGGAAATAGCGCATATTCTTCATCCTGACA  
 ACACCAGCCGATGGCACGACCTTATCGGTATAAAGAAAGTAGGACTGT  
 ATCAGGAAGATGCAGAAGCAATCACTCATCTTCACTGATGTTCAAAT  
 TAGTCCTTCTACGTCTCGTTAGTAGAAAGTGACTACAAGTTTA  
 CGATATAACATTGGCTTGTTGGTGTAAATTATGATAGACTTGAACAACTTGC  
 GCTATATGTAAGCGAAACCACCATTAATAACTATCTGAACCTGTTGAACG  
 TGTTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG  
 ACCATTAGACTCTCTTATAGCTAACCTTACCGAGGTGATCTCCTCC  
 CTATCTCAGCGCTTATTACAGTACTGGTGGCACTCAGCTTCCAATCT  
 GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGGTGA  
 CTGGCTCGTCCCTTATAATTGCATCCAAATGATTTAGAAGCAGCAAG  
 GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTCGTT  
 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA  
 TAAGGTTATATAACTCCCTCTTACCGGTGCTTTAATCCATGTTGGCCT

196/254

FIGURE 41C (P2)

701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA  
CTAGACGTGGTAGGATCGCATTAATGTGAACCTTATCAACCCCCCTCT

751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT  
GAAAGGTGACGTTAAGTTCTCAGATTGGTCCTCGGAAACGATCAGGTTA

801 TCAACTGCAAAGACGTAATGGTCCAAATTCAAGTGTGTACGATGTGAGTA  
AGTTGACGTTCTGCATTACCAAGGTTAACGTACACACATGCTACACTCAT

851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA  
ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACGCGTGGAGGTGGT

901 TCGTCACAGTTT  
AGCAGTGTCAAA

Linker Sequence:  
 CACGGCCCCGAGGGTTAACAGAGTGGGATTTATGAATCTGACGTATGGG  
 GTGCCGGGCTCCAAATTCTCACCCCTAAAATCTAGACTGCAGTACCC  
 AAGAGGCCATGCTCGTTAGTTCATGTCGAAGAGCCTCACACT  
 TTCTCCGGTACGAGCAAATCAAGTACAGCAACTCGGAGTGTGA

949 GC  
CG

951 TGATGTTGTATGGATCCTGAGCCCAGTGCGTATCGTAGGTGAAATG  
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC

1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
CAGATAACACAACATCCCTACCTTCAAGGTGTTGCCTTGCCTTAC

1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
GTCAACACCGGTACGTTAGATTGTCTACGTTAGTCGAGACCTGAAA

1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC

1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA  
CCATGTCAGGCCCTCAGATAACACTAGATACTAACGTTATGACGACGT

1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC  
TGACTACGGTGGCGACCGTTATACCTTACCTGGTAGTATTTAGG

1251 CAGATCTAGTCTAGTTAGCAGCGACATCAGGAAACAGTGGTACCAACAC  
GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTCACCATGGTGTG

197/254

**FIGURE 41C (P3)**

1301 TTACAGTGCAAACCAACATTATGCCGTTAGTCAGGGTTGGCTTCCCTACT  
AATGTCACGTTGGTTGAAATACGGCAATCAGTCCAACCGAAGGATGA

1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC

1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
GAACGTTCGTTATCACCTGTTACACCTATCTCCTGACATCGTCACTT

1451 AGGCTGAACAAACAGTGGCTCTTATGCAGATGGTTCAATACGTCCCTCAG  
TCCGACTTGGTGTACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC

1501 CAAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATAACGGAAACAGT  
GTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTGTCA

1551 TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA

1601 TCAAGAAATGATGGAACCATTAAATTGTATAGTGGATTGGTAGAT  
AGTTCTTACTACCTTGGTAAAATTAAACATATCACCTAACCAACATCTA

1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTACCCCTCTCCA  
CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT

1701 TGGTGACCAAACCAAATATGGTACCATTTGATAGACAGATTACT  
ACCACTGGTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA

1751 CTCTTGAGTGTGTGTCCGCCATGAAAATAGATGGCTAAATAAAAAA  
GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATTTT

1801 GGACATTGTAATTTGTAAGTGGACAGCAAGTTATATCGAATTCC  
CCTGTAACATTAAAACATTGACTTCCCTGTCGTTCAATATAGCTTAAGG

1851 TGCAG  
ACGTC

198/254

**FIGURE 41D**

**Amino acid sequence Comparison of Mutant Preproricin Linker  
region of MMP-11 (Stromelysin-3) to Wild Type**

Wild type ricin linker:            A chain- S L L I R P V V P N F N -B chain

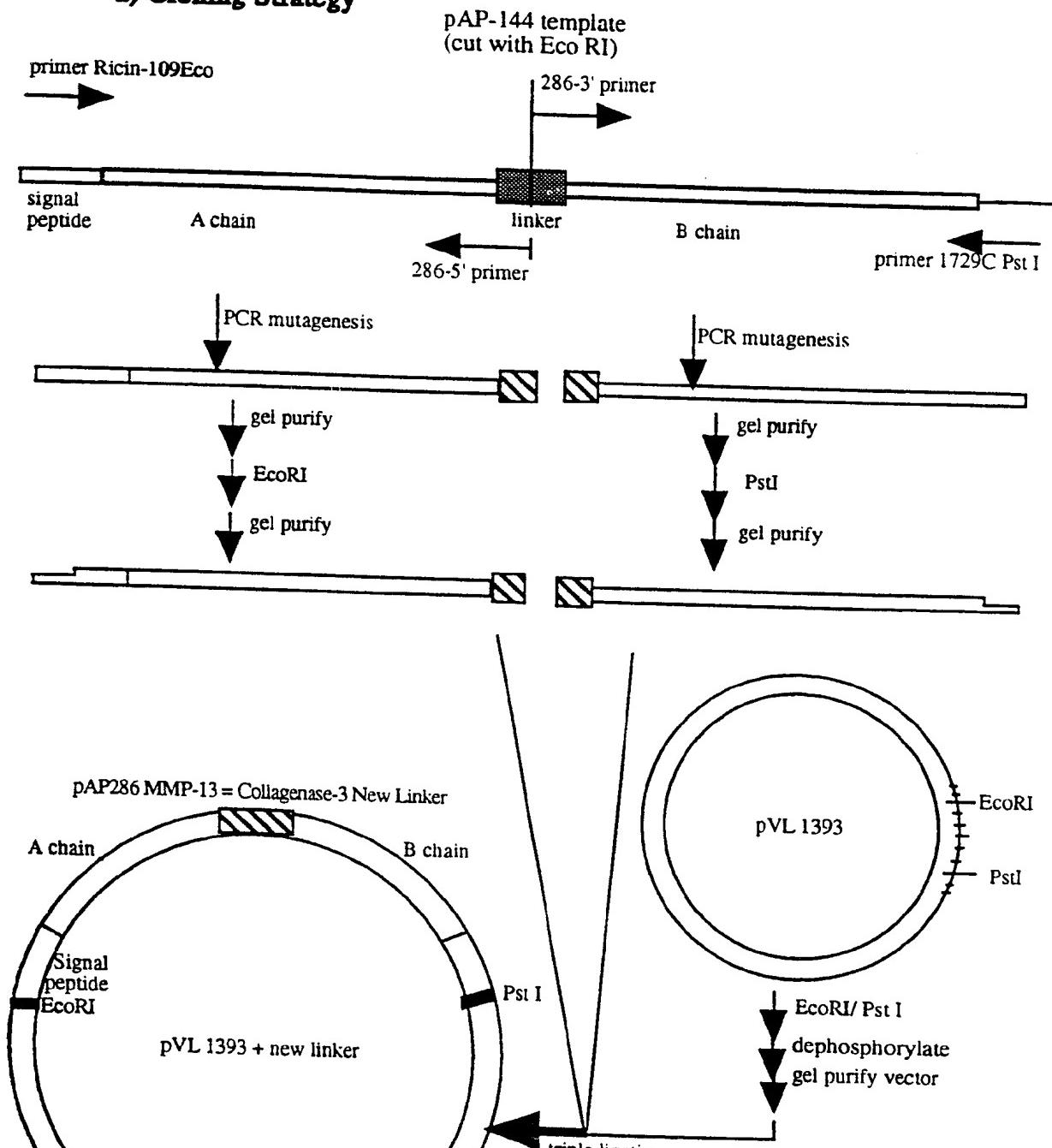
pAP-284 (MMP-11) linker:

A chain- H G P E G L R V G F Y    E S D V M G R G H A R L V H V E E P H T -B chain

199/254

**FIGURE 42A**

**PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393**

**a) Cloning Strategy**

200/254

**FIGURE 42B****Sequence of MMP-13 = Collagenase-3 Linker Region****WT preprocin linker**

primer 286-3'  
5' - GGTCAACGAGGCATTGTCGCTGATGTTGT - 3'  
\*\*\*\*\* \* \*\*\*\*\* \*\*\*  
-----TCTTGCTTATAAGGCCA|GTGGTACCAAATTAAAT-----  
-----AGAAACGAATATTCCGGT|CACCATGGTTAAAATTA-----  
\*\*\*\*\* \* \*\*\*\*\* \*  
3' -AGCAGTGTCAAACCTGGAGTCCCCAACGA -5'  
primer 286-5'

1) PCR mutagenesis

2) Ligate with pVL1393

**pAP 286 linker  
(MMP-13 variant)**

-----GGACCTCAGGGGCTTGCT|GGTCAACGAGGCATTGTC-----  
-----CCTGGAGTCCCCAACGA|CCAGTTGCTCCGTAAACAG-----

201/254

**FIGURE 42C (P1)**

Sequence of pAP286 insert

10	20	30	40	50
1 GAATTCATGAAACCAGGGAGGAAATACTATTGTAATATGGATGTATGCAGT CTTAAGTACTTGGCCCTCCTTATGATAACATTACACATACGTCA				
51 GGCAACATGGCTTGTGGATCCACCTCAGGGTGGTCTTCACATTAG CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC				
101 AGGATAACAACATATTCCCCAAACAATACCAATTATAAACTTACCA TCCTATTGTTGTATAAGGGTTGTTATGGGTTAATATTGAAATGGTGT				
151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTCGCC CGCCCACGGTGACACGTTCGATGTGTTGAAATAGTCTCGACAAGCGCC				
201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATAACCAGTGGCAA AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT				
251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACCTCA TGTCTCAACCAACGGATATTGGTGCCTAAATCAACTTGAGAGT				
301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT				
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTCTTCATCCTGACA ACACCAGCCGATGGCACGACCTTATCGGTATAAAGAAAGTAGGACTGT				
401 ATCAGGAAGATGCAGAAGCAACTCATCTTCACTGATGTTCAAAAT TAGTCCTCTACGTCTCGTTAGTAGAAAAGTAGACTACAAGTTTA				
451 CGATATAACATTGCCTTGGTGGTAATTATGATAGACTGAAACAACCTGC GCTATATGTAAGCGAAACCACCATTAATAACTATCTGAACCTGTTGAACG				
501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG ACCATTAGACTCTCTTATAGCTAACCTTACCAAGGTGATCTCCTCC				
551 CTATCTCAGCGCTTATTACAGTACTGGTGGCACTCAGCTCCA GATAGAGTCGGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA				
601 CTGGCTCGTCCTTATAATTGCATCCAAATGATTCAGAAGCAGCAAG GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTCGTT				
651 ATTCCAATATATTGAGGGAGAAATGCCACGAGAATTAGGTACAACCGGA TAAGGTTATATAACTCCCTCTTACCGTGCTTTAATCCATGTTGGCCT				
701 GATCTGCACCAAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA				

202/254

**FIGURE 42C (P2)**

CTAGACGTGGCTAGGATCGCATTAATGTGAACCTCTTATCAACCCCTCT  
 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT  
     GAAAGGTGACGTTAAGTCTCAGATTGGTCCTCGGAAACGATCAGGTTA  
 801 TCAACTGCAAAGACGTAATGGTCAAATTCAAGTGTGTACGATGTGAGTA  
     AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT  
 851 TATTAATCCCTATCATAGCTCTCATGGGTATAGATGCGCACCTCCACCA  
     ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT  
 901 TCGTCACAGTTGGACCTCAGGGGCTTGCTGGTCAACGAGGCATTGTCGC  
     AGCAGTGTCAAACCTGGAGTCCCCAACGACCAGTTGCTCCGTAACAGCG  
 951 TGATGTTGTATGGATCCTGAGCCCAGTGCATCGTAGGTGAAATG  
     ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
     CAGATACACAACATACCAATCCCTACCTCTAAGGTGTTGCCCTTGCCTTAC  
 1051 CAGTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
     GTCAACACCGGTACGTTAGATTATGTCTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
     CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAAACTGCTGCA  
     CCATGTCAGGCCCTCAGATAACACTAGATAACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC  
     TGACTACGGTGGCGACCGTTATACCCCTATTACCTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
     GTCTAGATCAGATCAAATCGCTGTAGTCCCTGTCACCATGGTGTG  
 1301 TTACAGTGCAAACCAACATTATGCCGTTAGTCAAGGTTGGCTTCTACT  
     AATGTCACGTTGGTTGTAATACGGCAATCAGTCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
     TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
     GAACGTTCGTTATCACCTGTTACACCTATCTCCTGACATCGTCACCTT  
 1451 AGGCTGAACAAACAGTGGCTCTTATGCAGATGGTCAATACTGCTCAG  
     TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGATTCTAATACGGAAACAGT

203/254

**FIGURE 42C (P3)**

GTTTGGCTCTATTACCGGAATGTTCACTAAGATTATATGCCCTTGTC

1551 TGTAAAGATCCTCTCTGTGGCCCTGCATCCTCTGCCAACGATGGATGT  
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACC GGTTGCTACCTACA1601 TCAAGAATGATGGAACCATTTAAATTGTATAAGTGGATTGGTAGAT  
AGTTCTTACTACCTTGGTAAAATTAAACATATCACCTAACCAATCTA1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTACCCCTCCA  
CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT1701 TGGTGACCCAAACCAAATATGGTTACCATTTTGTATAAGACAGATTACT  
ACCACTGGGTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA1751 CTCTTGCAGTGTGTGTGCCTGCCATGAAAATAGATGGCTTAAATAAAAAA  
GAGAACGTCACACACACAGGACGGTACCTTATCTACCGAATTATTTT1801 GGACATTGTAAATTTGTAAGTGAAAGGACAGCAAGTTATATCGAATTCC  
CCTGTAACATTAAAACATTGACTTCTGTCGTTCAATATAGCTTAAGG1851 TGCAG  
ACGTC

204/254

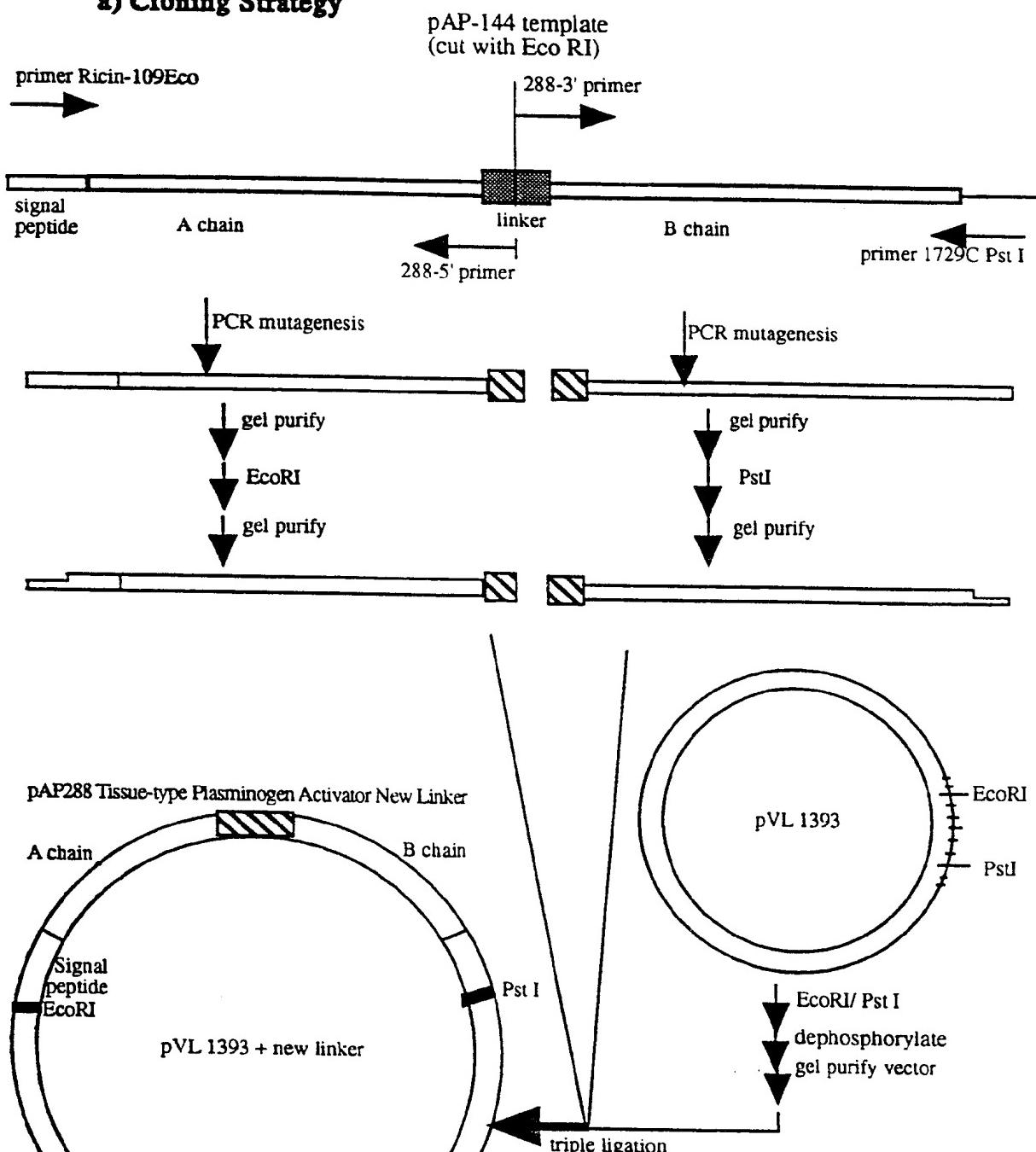
**FIGURE 42D**

**Amino acid sequence Comparison of Mutant Preproricin Linker  
region of MMP-13 (Collagenase-3) to Wild Type**

Wild type ricin linker:            A chain- S L L I R P V V P N F N -B chain

pAP-286 (MMP-13) linker:        A chain- G P Q G L A G Q R G I V -B chain

205/254

**FIGURE 43A****PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

206/254

**FIGURE 43B****Sequence of Tissue-type Plasminogen Activator (tPA) Linker Region****WT preprocin linker**

primer 288-3'

5' - GGTCTAAAGCTCTTGAAGCTGATGTTGT - 3'  
\*\*\*\*\* \* \* \*

-----TCTTGCTTATAAGGCCA|GTGGTACCAAATTAAAT-----

-----AGAAACGAATATTCCGGT|CACCATGGTTAAAATTA-----

\*\*\*\*\* \* \*\*\*\*\*

3' - AGCAGTGTCAAACCGCCTAGACCCGTTCC - 5'

primer 288-5'

1) PCR mutagenesis

2) Ligate with pVL1393

**pAP 288 linker  
(tPA variant)**

----- GGCGGATCTGGGCAAAGG|GGTCGTAAAGCTCTTGA -----

----- CCGCCTAGACCCGTTCC|CCAGCATTGAGAACTT -----

207/254

**FIGURE 43C (P1)**

Sequence of pAP288 insert

10	20	30	40	50
1				

1 GAATTCATGAAACGGGGAGGAATACTATTGTAATATGGATGTATGCAGT  
CTTAAGTACTTGGCCCTCCTTATGATAACATTATACCTACATACGTCA

51 GGCACACATGGCTTGTGATCCACCTCAGGGTGGTCTTCACATTAG  
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAA

101 AGGATAACAACATATTCCCCAAACAATAACCAATTATAAACTTACCA  
TCCTATTGTTGTATAAGGGTTGTTATGGGTTAATATTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTTCGCG  
CGCCCACGGTGACACGTTGATGTGTTGAAATAGTCTCGACAAGCGCC

201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATAACCAGTGTGCCAA  
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACTCTCA  
TGTCTAACCAACGGATATTGGTTGCCAAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTACCAATGCATA  
TTAGTACGTCTCGAAAGACAATGTAATCGCAGACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTCTTCATCCTGACA  
ACACCAGCCGATGGCACGACCTTATCGGTATAAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAACAACTCACTCATCTTCACTGATGTTAAAAT  
TAGTCCTTCTACGTCTCGTTAGTGAGTAGAAAGTGAACATCAAGTTTA

451 CGATATACATTCGCTTGGTGGTAATTATGATAGACTTGAACAACTTGC  
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACATTGTTGAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG  
ACCATTAGACTCTCTTATAGCTAACCCCTTACCAAGGTGATCTCCTCC

551 CTATCTCAGCGCTTATTACAGTACTGGTGGCACTCAGCTTCCA  
GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTCTTATAATTGCATCAAATGATTTCAGAACAGCAAG  
GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTCGTT

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA  
TAAGGTTATATAACTCCCTTTACCGGTGCTTTAATCCATGTTGGCCT

701 GATCTGCACCAAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA

208/254

**FIGURE 43C (P2)**

CTAGACGTGGCTAGGATCGCATTAATGTGAACCTTATCAACCCCTCT  
 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT  
GAAAGGTGACGTTAAGTTCTCAGATTGGTCCTCGGAAACGATCAGGTAA  
 801 TCAAATGCAAAGACGTAATGGTCCAAATTCAAGTGTGTACGATGTGAGTA  
AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT  
 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA  
ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT  
 901 TCGTCACAGTTGGCGGATCTGGCAAAGGGGTCGTAAAGCTTGAAGC  
AGCAGTGTCAAACCGCTAGACCCGTTCCCCAGCATTGAGAACTTCG  
 951 TGATGTTGTATGGATCCTGAGCCCATACTGCCTAGTGTAGGTGAAATG  
ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
CAGATACACAACATACAAATCCCTACCTTCTAAGGTGTTGCCCTTGCCTTAT  
 1051 CAGTTGTGGCCATGCAAGTCTAACAGATGCAAATCAGCTCTGGACTTT  
GTCAACACCGGTACGTTACGATTATGTCTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAACAGATGGAAAGTGTAACTACTTACG  
CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAAACTGCTGCA  
CCATGTCAGGCCCTCAGATAACACTACTAGATAACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC  
TGACTACGGTGGCGACCGTTATACCCATTACCTTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
GTCTAGATCAGATCAAATCGTCGTGTAGTCCCTGTACCATGGTGT  
 1301 TTACAGTGCACAAACACATTATGCCGTTAGTCAAGGTGGCTTCTACT  
AATGTCACGTTGGTTGTAATACGGCAATCAGTTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATGTTGGCTATATGGTCTGTG  
TTATTATGTGTTGGAAAACAATGTTGGTAACAAACCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAG  
GAACGTTCGTTATCACCTGTTACACCTATCTCCTGACATCGTCACCTT  
 1451 AGGCTGAACAAACAGTGGCTTTATGCAGATGGTCAATACGTCCCTCAG  
TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGATTCTAATATACGGAAACAGT

209/254

**FIGURE 43C (P3)**

GTGTTGGCTCTATTAACGGAATGTTCACTAAGATTATGCCCTTGTC  
1551 TGTAAAGATCCTCTCTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
ACAATTCTAGGAGAGAACACCAGGACGTAGGAGACCGGTTGCTACCTACA  
1601 TCAAGAATGATGGAACCATTAAATTGTATAGTGGATTGGTGTAGAT  
AGTTCTTACTACCTTGGTAAAATTAAACATATCACCTAACCAATCTA  
1651 GTGAGGCGATCGGATCCGAGCCTAAACAAATCATCTTACCCCTCTCCA  
CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
1701 TGGTGACCCAAACCAAATATGGTTACCATATTGTATAGACAGATTACT  
ACCACTGGGTTGGTTATAACCAATGGTAATAAAACTATCTGTCTAATGA  
1751 CTCTTGCAGTGTGTGTGCCTGCCATGAAAATAGATGGCTAAATAAAAAA  
GAGAACGTCACACACACAGGACGGTACTTTATCTACCGAATTATT  
1801 GGACATTGTAATTTGTAAGTGAAGGACAGCAAGTTATATCGAATTCC  
CCTGTAACATTAAAACATTGACTTCCCTGTCGTTCAATATAGCTTAAGG  
1851 TGCAG  
ACGTC

Total number of bases is: 1855.

Sequence name: PAP288

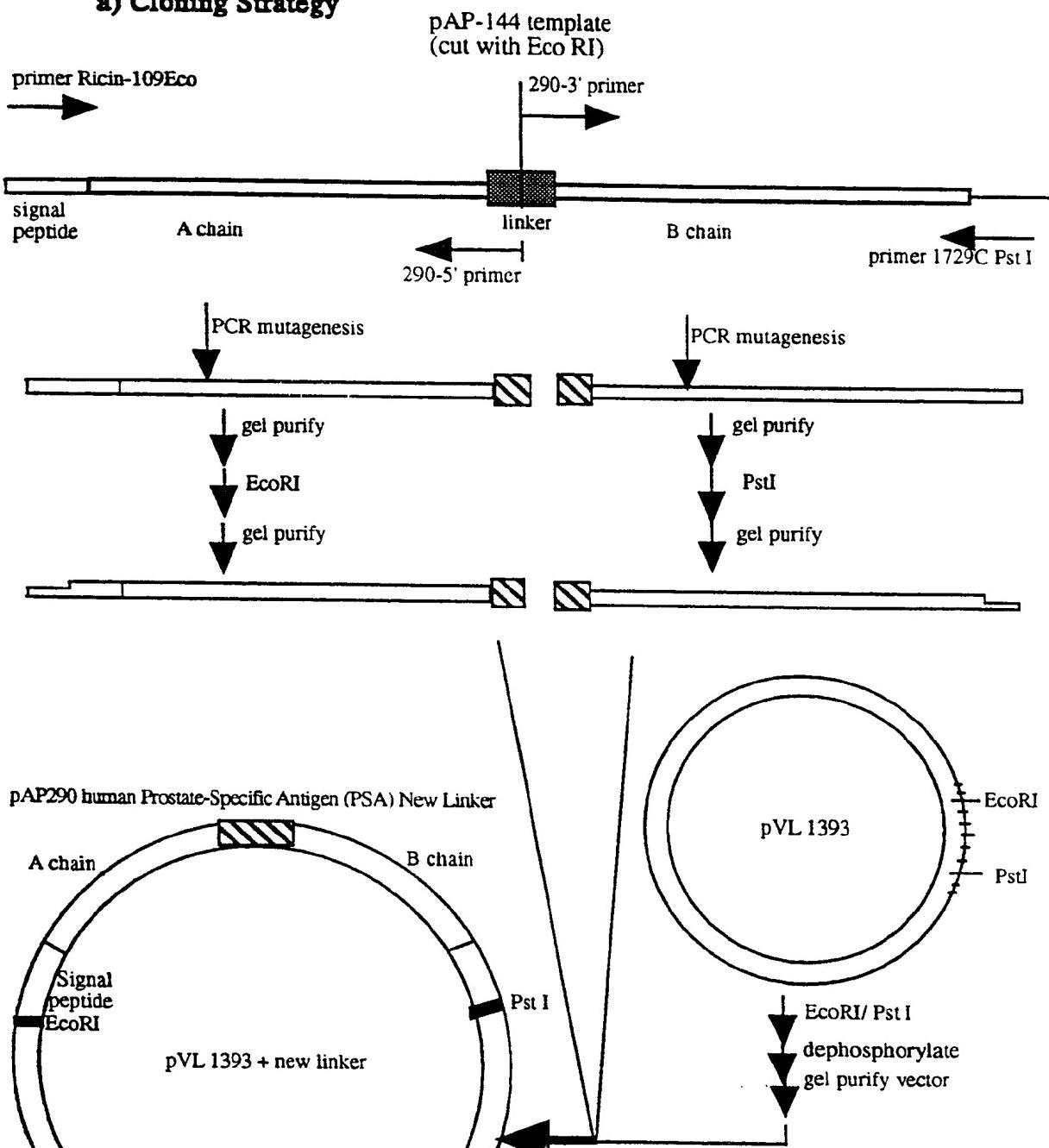
210/254

**FIGURE 43D**

**Amino acid sequence Comparison of Mutant Preproricin Linker  
region of Tissue-type Plasminogen Activator (tPA) to Wild Type**

Wild type ricin linker:                   A chain- S L L I R P V V P N F N -B chain  
pAP-288 (tPA) linker:                   A chain- G G S G Q R G R K A L E-B chain

211/254

**FIGURE 44A****PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

212/254

**FIGURE 44B****Sequence of human Prostate-Specific Antigen (PSA) Linker Region****WT preprocin linker**

primer 290-3'  
5' - TCTTCCGATATTTAATGCTGATGTTGT - 3'  
\*\*\*\*\* \* \*

-----TCTTGCTTATAAGGCCA|GTGGTACCAAATTTAAT-----  
-----AGAAACGAATATTCCGGT|CACCATGGTTAAAATTA-----  
\*\*\*\*\* \* \*  
3'-AGCAGTGTCAAAAGAACAGTCGAGAAGAG - 5'  
primer 290-5'

1) PCR mutagenesis

2) Ligate with pVL1393

**pAP 290 linker  
(PSA variant)**

-----TCTTGTCAGCTCTCTC|TCTTCCGATATTTAAT-----  
-----AGAAACAGTCGAGAAGAG|AGAAGGCTATAAAAATTA -----

213/254

**FIGURE 44C (P1)**

Sequence of pAP290 insert

10	20	30	40	50

1 GAATTCATGAAACCGGGAGGAAATACTATTGTAAATATGGATGTATGCAGT  
 CTTAAGTACTTTGCCCTCCTTATGATAACATTACACATACACGTCA  
 51 GGCAACATGGCTTTGGATCCACCTCAGGGTGGCTTCACATTAG  
 CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGAATC  
 101 AGGATAACAAACATATTCCCCAAACAATACCAATTATAAACTTACCA  
 TCCTATTGTTGATAAGGGTTGTTATGGGTTAATATTGAAATGGTGT  
 151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTCGCC  
 CGCCCACGGTGACACGTTGATGTGTTGAAATAGTCTCGACAAGCGCC  
 201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATACCAAGTGTGCCA  
 AGCAAATTGTTGACCTCGACTACACTCTGTAATATGGTCACAAACGGTT  
 251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACCTCTCA  
 TGTCTCAACCAAACGGATATTGGTGCCTAAATCAAACCTTGAGAGT  
 301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA  
 TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT  
 351 TGTGGTCGGTACCGTGCTGGAAATAGCGCATATTCATCCTGACA  
 ACACCAGCCGATGGCACGACCTTATCGCGTATAAGAAAGTAGGACTGT  
 401 ATCAGGAAGATGCAGAAAGCAATCACTCATCTTCACTGATGTTAAAAT  
 TAGTCCTCTACGTCTCGTTAGTAGAAAGTAGACTACAAGTTTA  
 451 CGATATACATTGCCCTTGGTGGTAATTATGATAGACTGAAACAACCTGC  
 GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACCTGTTGAACG  
 501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG  
 ACCATTAGACTCTTTATAGCTAACCTTACCAAGGTGATCTCCTCC  
 551 CTATCTCAGCGCTTATTACAGTACTGGTGGCACTCAGCTTCCA  
 ACTGATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA  
 601 CTGGCTCGTCTTATAATTGCATCCAAATGATTCAGAAGCAGCAAG  
 GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTCGTT  
 651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA  
 TAAGGTTATATAACTCCCTCTTACCGTGCTCTTAATCCATGTTGGCCT  
 701 GATCTGCACCAAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA

214/254

**FIGURE 44C (P2)**

CTAGACGTGGCTAGGATCGCATTAATGTGAACCTTATCAACCCCTCT  
 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT  
     GAAAGGTGACGTTAAGTCTCAGATTGGTCCTCGAAACGATCAGGTTA  
 801 TCAACTGCAAAGACGTAATGGTCAAATTCAAGGAGCCTTGCTAGTCCAAT  
     AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT  
 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA  
     ATAATTAGGGATAGTATCGAGAGTACACATATCTACGCGTGGAGGTGGT  
 901 TCGTCACAGTTCTTCAGCTCTCTCTCTCCGATATTTTAATGC  
     AGCAGTGTCAAAAGAAACAGTCGAGAAGAGAAGAGCTATAAAATTACG  
 951 TGATGTTGTATGGATCCTGAGCCCAGTGCATCGTAGGTCGAAATG  
     ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTGATGTTAGGGATGGAAGATTCCACAACGAAACGCAATA  
     CAGATACACAACATACCAATCCCTACCTTCAAGGTGTTGCCTTGCCTTAT  
 1051 CAGTTGTGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
     GTCAACACCAGTACGTTAGATTATGTCTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
     CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAAACTGCTGCA  
     CCATGTCAGGCCCTCAGATACTACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCGCTGGCAAATATGGGATAATGGAACCACATCAAATCC  
     TGACTACGGTGGCGACCGTTATACCCATTACCTTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
     GTCTAGATCAGATCAAATCGCTGTAGTCCCTGTCACCATGGTGTG  
 1301 TTACAGTGCACAAACACATTATGCCGTTAGTCAAGGTTGGCTTCTACT  
     AATGTCACGTTGGTTGAAATACGGCAATCAGTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
     TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
     GAACGTTCGTTATCACCTGTCATAACCTATCTCCTGACATCGTCACCTT  
 1451 AGGCTGAACAAACAGTGGCTTTATGCAGATGGTCAATACTGCCTCAG  
     TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATACGGAAACAGT

215/254

**FIGURE 44C (P3)**

GTTCGGCTCTATTAACGGAATGTTACTAAGATTATGCCCTTGTCA  
1551 TGTAAAGATCCTCTTGTGCCCTGCATCCTCTGGCCAACGATGGATGT  
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCAGGTGCTACCTACA  
1601 TCAAGAATGATGGAACCATTTAAATTGTATAGTGGATTGGTAGAT  
AGTTCTTACTACCTGGTAAAATTAAACATATCACCTAACCAACATCTA  
1651 GTGAGGCGATCGGATCCGAGCCTAAACAAATCATTCTTACCCCTCTCCA  
CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
1701 TGGTGACCCAAACCAAATATGGTTACCATTATTTGATAGACAGATTACT  
ACCACTGGGTTGGTTATACCAATGGAATAAAACTATCTGTCTAATGA  
1751 CTCTTGCAGTGTGTGTGCCTGCCATGAAAATAGATGGCTAAATAAAAAA  
GAGAACGTACACACACAGGACGGTACTTTATCTACCGAATTATTTT  
1801 GGACATTGTAATTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC  
CCTGTAACATTTAAACATTGACTTCCGTCAATATAGCTTAAGG  
1851 TGCAG  
ACGTC

Total number of bases is: 1855.

Sequence name: PAP290

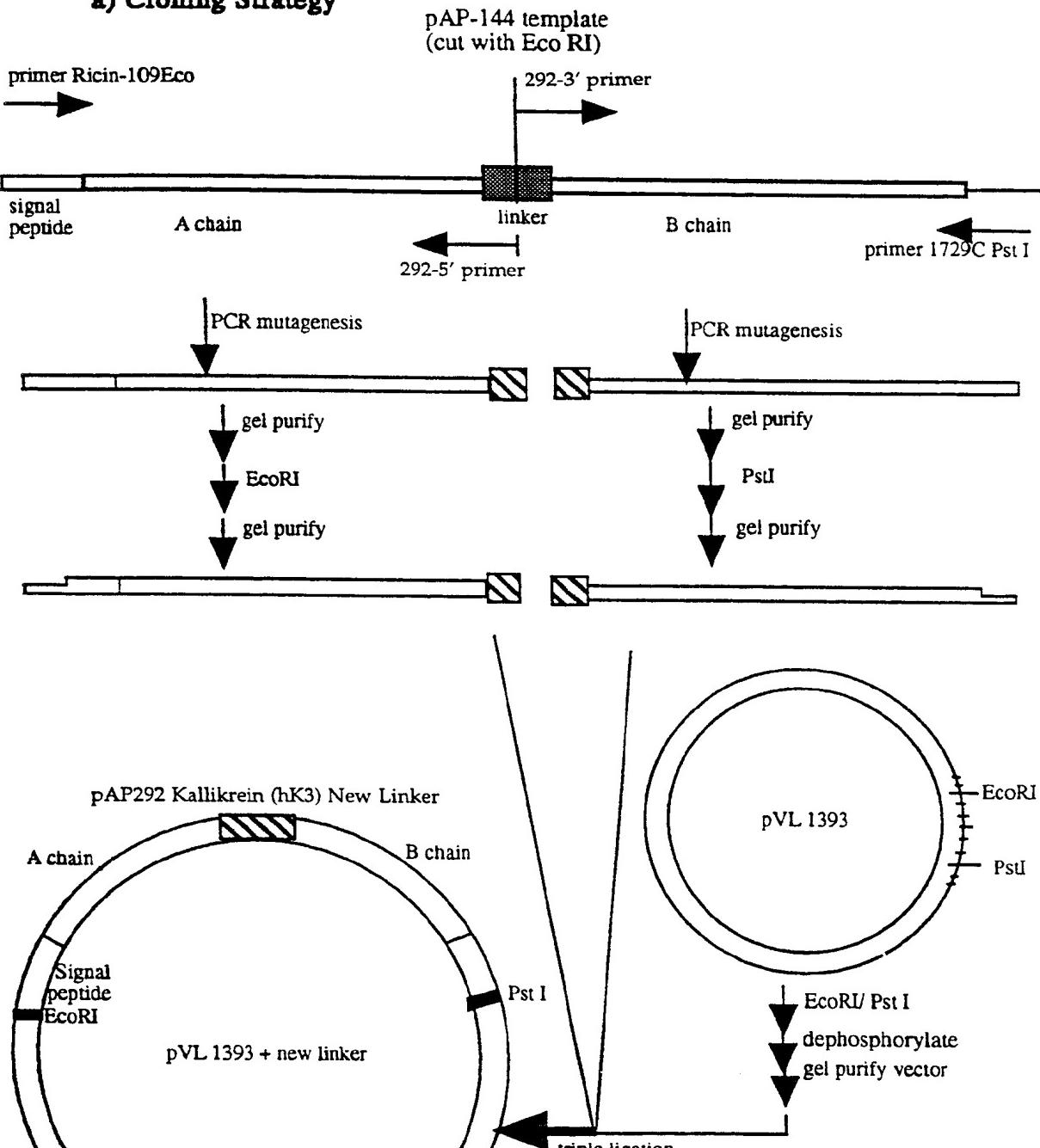
216/254

**FIGURE 44D**

**Amino acid sequence Comparison of Mutant Preproricin Linker  
region of human Prostate-Specific Antigen (PSA) to Wild Type**

Wild type ricin linker:                    A chain- S L L I R P V V P N F N -B chain  
pAP-290 (PSA) linker:                    A chain- S L S A L L S S D I F N -B chain

217/254

**FIGURE 45A****PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

218/254

**FIGURE 45B****Sequence of Kallikrein (hK3) Linker Region****WT preprocin linker**

primer 292-3'

5' - ATTATCGGTGGCTTTAATGCTGATGTTGT - 3'  
\* \* \* \*\*\*\*\*-----  
TCTTTGCCTATAAGGCCA | GTGGTACCAAATTAAAT-----  
AGAACGAATATTCCGGT | CACCATGGTTAAAATTA

\* \* \* \*\*\*\*

3' - AGCAGTGTCAAAAGAAACGGATCTAAATT - 5'

primer 292-5'

1) PCR mutagenesis

2) Ligate with pVL1393

**pAP 292 linker**

(Kallikrein variant)

-----  
TCTTTGCCTAGATTAAA | ATTATCGGTGGCTTTAAT-----  
AGAACACGGATCTAAATT | TAATAGCCACCGAAATTA

219/254

**FIGURE 45C (P1)****Sequence of pAP292 insert**

10	20	30	40	50

1 GAATTCATGAAACCGGGAGGAAATACTATTGTAATATGGATGTATGCAGT  
CTTAAGTACTTTGCCCTCCTTATGATAACATTACACATACGTCA

51 GGCACACATGGCTTGTGGATCCACCTCAGGGTGGTCTTCACATTAG  
CCGTTGTACCGAAACAAAACCTAGGTGGAGTCCCACCAGAAAGTGTAA

101 AGGATAACAACATATTCCCCAAACAATAACCAATTATAAACTTTACCA  
TCCTATTGTTGTATAAGGGGTTGTTATGGGTTAATATTGAAATGGTGT

151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTTATCAGAGCTGTTGCCGG  
CGCCCACGGTGACACGTTCGATGTGTTGAAATAGTCTCGACAAGCGCC

201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATAACCAGTGTGCCAA  
AGCAAATTGTTGACCTCGACTACACTCTGACTATATGGTCACAACGGTT

251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACTCTCA  
TGTCTAACCAACGGATATTGGTTGCCAAATAAAATCAACTTGAGAGT

301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA  
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT

351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTCTCATCCTGACA  
ACACCAGCCGATGGCACGACCTTATCGCGTATAAGAAAGTAGGACTGT

401 ATCAGGAAGATGCAGAAGCAATCACTCATTTCACTGATGTTAAAAT  
TAGTCCTCTACGTCTCGTTAGTGAGTAGAAAGTGAACACTAACGTTA

451 CGATATACATTGCCCTTGGTGGTAATTATGATAGACTTGAACAACTTGC  
GCTATATGTAAGCGGAAACCACCAATTAAACTATCTGAACCTGTTGAACG

501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGAGG  
ACCATTAGACTCTCTTATAGCTAACCTTACCAAGGTGATCTCCTCC

551 CTATCTCAGCGCTTTATTACAGTACTGGTGGCACTCAGCTCCA  
GATAGAGTCGCAGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA

601 CTGGCTCGTCCTTATAATTGCATCAAATGATTCAGAAGCAGCAAG  
GACCGAGCAAGGAAATATTAACGTAGGTTACTAAAGTCTCGTCGTTC

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA  
TAAGGTTATATAACTCCCTCTTACCGCGTGTCTTAATCCATGTTGGCCT

701 GATCTGCACCAAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA

220/254

FIGURE 45C (P2)

CTAGACGTGGTCTAGGATCGCATTAAATGTGAACCTTATCAACCCCTCT  
 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT  
       GAAAGGTGACGTTAAGTCTCAGATTGGTCCTCGAAACGATCAGGTTA  
 801 TCAACTGCAAAGACGTAATGGTCAAATTCAAGTGTGTACGATGTGAGTA  
       AGTTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT  
 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA  
       ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACGCGTGGAGGTGGT  
 901 TCGTCACAGTTTCTTGCTAGATTAAAATTATCGGTGGCTTAATGC  
       AGCAGTGTCAAAAGAACGGATCTAAATTAAATAGCCACCGAAATTACG  
 951 TGATGTTGATGGATCCTGAGCCCAGTGCATCGTAGGTCGAAATG  
       ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
       CAGATAACAAACTACAATCCCTACCTCTAAGGTGTTGCCCTTGCATTAT  
 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
       GTCAACACCGGTACGTTCAAGATTATGTCACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
       CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCGGGAGTCTATGTGATGATCTATGATTGCAAAACTGCTGCA  
       CCATGTCAGGCCCTCAGATAACTACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCACATCATAAATCC  
       TGACTACGGTGGCGACCGTTATACCCATTACCTTGGTAGTATTAGG  
 1251 CAGATCTAGCTAGTTAGCAGCGACATCAGGAACAGTGGTACACAC  
       GTCTAGATCAGATCAAATCGCGCTGTAGTCCCTGTACCATGGTGTG  
 1301 TTACAGTGCAAACCAACATTATGCCGTTAGTCAAGGTTGGCTTCTACT  
       AATGTCACGTTGGTGTAAATACGGCAATCAGTCCAAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGCTGTG  
       TTATTATGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGGACTGTAGCAGTGAAA  
       GAACGTTCGTTATCACCTGTCACTATCTCTGACATCGTCACTTT  
 1451 AGGCTGAACAAACAGTGGCTTTATGCAGATGGTCAATACGTCTCAG  
       TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCCTACAAGTGATTCTAATATACGGAAACAGT

221/254

**FIGURE 45C (P3)**

GTTTGGCTCTATTACCGAATGTCACTAAGATTATGCCCTTGTC  
1551 TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA  
1601 TCAAGAATGATGGAACCATTAAATTGTATAGTGGATTGGTAGAT  
AGTTCTTACTACCTTGGTAAATTAACATATCACCTAACCAACATCTA  
1651 GTGAGGCGATCGGATCCGAGCCTAAACAAATCATTCTTACCCCTCTCCA  
CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
1701 TGGTGACCCAAACCAAATATGGTTACCATTATTGATAAGACAGATTACT  
ACCACTGGGTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA  
1751 CTCTTGCAGTGTGTGTGCCTGCCATGAAAATAGATGGCTAAATAAAA  
GAGAACGTACACACACAGGACGGTACTTTATCTACCGAATTATTTT  
1801 GGACATTGTAATTTGTAAGTGAAAGGACAGCAAGTTATATCGAATTCC  
CCTGTAACATTAAAACATTGACTTCCTGTCGTTCAATATAGCTTAAGG  
1851 TGCAG  
ACGTC

Total number of bases is: 1855.

Sequence name: PAP292

222/254

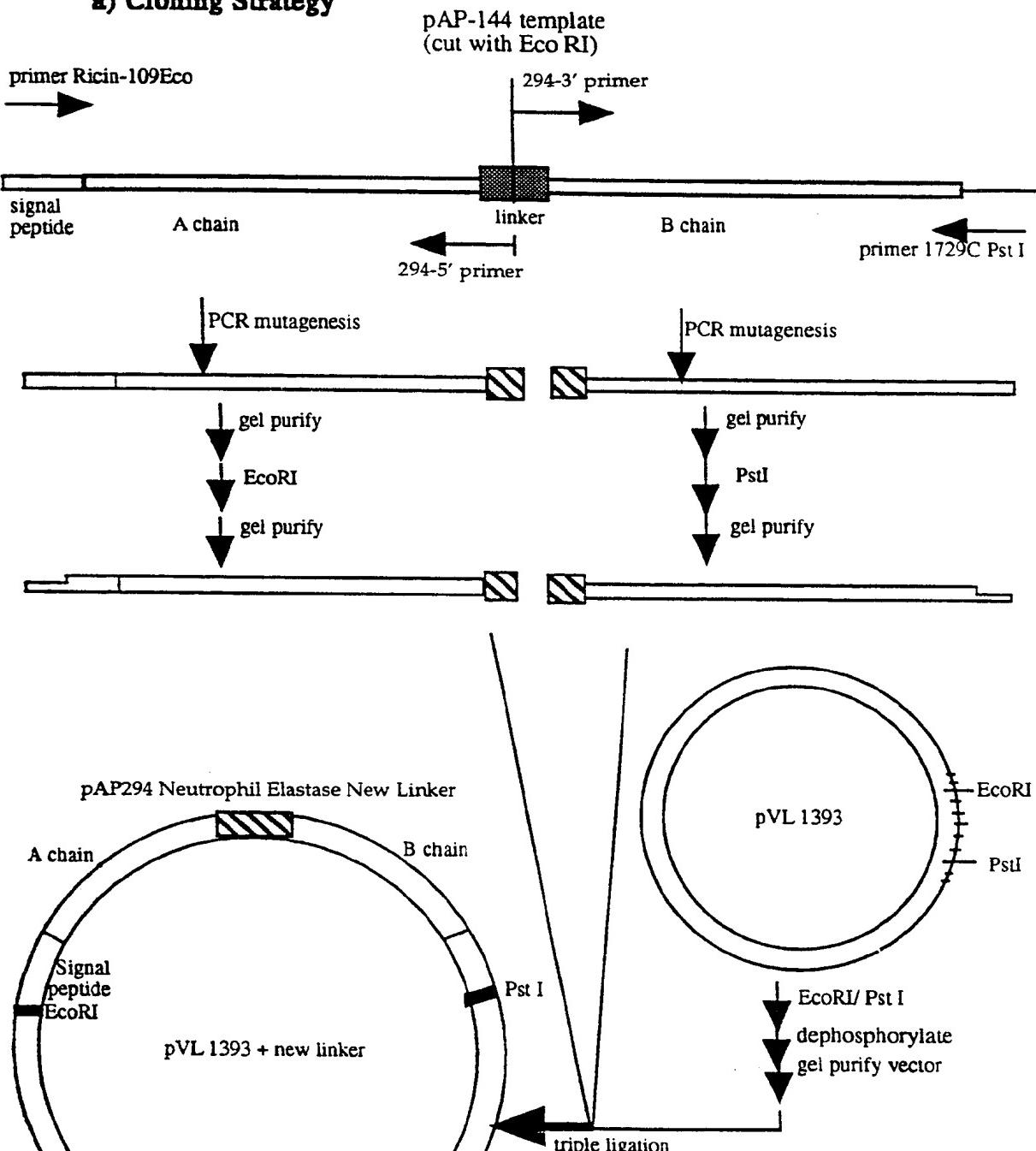
**FIGURE 45D**

Amino acid sequence Comparison of Mutant Preproricin Linker  
region of Kallikrein (hK3) to Wild Type

Wild type rycin linker: A chain- S L L I R P V V P N F N -B chain

pAP-292 (hK3) linker: A chain- S L P R F K I I G G F N -B chain

223/254

**FIGURE 46A****PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

224/254

**FIGURE 46B**

## Sequence of Neutrophil Elastase Linker Region

**WT preprocin linker**

primer 294-3'

5' - GTTCCTGGTAATTTAATGCTGATGTTGT - 3'  
\*\* \*\*\*\*\*

```
-----TCTTTGCTTATAAGGCCA|GTGGTACCAAAATTAAAT-----
-----AGAAACGAATATTCCGGT|CACCATGGTTAAAATTA-----
```

\*\*\* \*\*\* \*

3' - AGCAGTGTCAAAAGAACGAAACCGTAACGA - 5'

primer 294-5'

1) PCR mutagenesis

2) Ligate with pVL1393

**pAP 294 linker**

(Neutrophil elastase variant)

```
-----TCTTTGCTTGGCATTGCT|GTTCCTGGTAATTTAAT-----
-----AGAAACGAACCGTAACGA|CAAGGACCATTAAAATTA-----
```

225/254

FIGURE 46C (P1)

## Sequence of pAP294 insert

10	20	30	40	50
1	GAATT	CATGAA	ACCGGGAGG	AAATACTATT
	TTT	GGATCC	CACCTCAGGGTGG	TTCACATTAG
51	GGCAACATGG	CTTGTGG	GTCTTG	GGTACACATTAG
	CGTTGTACCG	AAAC	ACCTAGGTGG	GTCCCACCAGAAAGTGTAA
101	AGGATAACA	ACATATT	CCCCAAACA	ATACCCAATTATAA
	ACTTTACC	ACA	ACTTAC	TTACCA
151	GC	GGGTGCC	ACTGTGCAA	AGCTACACAA
	CGCC	ACGGTGAC	ACGTTCGAT	GTGTTGAAATAGTCTCGAC
201	TCGTTAACAA	CTGGAGCTG	ATGTGAGACATG	ATACCAACTGTGTTGCCAA
	AGCAAATTGTTGAC	CTCGACTAC	ACTCTGTACT	ATATGGTCACAACGGTT
251	ACAGAGTTGG	TTGCCTATAA	ACCAACGGTT	ATTTAGTTGAACTCTCA
	TGTCTCAAC	AAACGG	ATATTGGT	GCCAAATAAAATCAACTGAGAGT
301	AATCATGCAGAG	CTTCTGTT	ACATTAGCGCTGG	ATGTCACCAATGCATA
	TTAGTACG	TCTCGA	AGACAA	ATGTAATCGCGACCTACAGTGGTTACGTAT
351	TGTGGTCGG	CTACCGTG	CTGGAAATAGCGC	ATATTCTTCATCCTGACA
	ACACCAGCCG	ATGGCAC	CTTATCGCGT	ATAAAAGAAAGTAGGACTGT
401	ATCAGGAAGATG	CAGAAGCA	ATCACTCAT	CTTTCACTGATGTTCAAAAT
	TAGCCTTCTAC	GTCTCGTT	AGTAGT	GAGTAGAAAAGTGA
451	CGATATACATT	CGCCTTGGT	GGTAATTATG	GATAGACTGAAACAAC
	TGCTATATG	TAAGCGGAA	ACCACCA	TTAAACTATCTGAAC
501	TGGTAATCTGAG	AGAGAAAATATG	GAGTTGGAAATGGT	CCACTAGAGGGAGG
	ACCATTAGACT	CTCTTT	TAGCTCAAC	CCCTTACCAAGGTGATCTCCTCC
551	CTATCTCAGCG	CTTATTATTAC	AGTACTGGTGG	CACTCAGCTTCAA
	GATAGAGTCG	CGAAATAATA	ATGT	CATGACCACCGTGAGTCGAAGGTTGA
601	CTGGCTCG	TTCTTATAATTG	CATCCAA	ATGATTTCAGAAGCAGCAAG
	GACCGAGCAAGG	AAATAAAAC	GTAGGTTACTAAAGT	CTTCGTCGTT
651	ATTCCAATATATT	GAGGGAGAAATG	CGCACGAGA	ATTAGGTACAACCGGA
	TAAGGTTATATA	ACTCC	CTTACGCGT	GCTCTTAATCCATGTTGGCCT
701	GATCTGCACC	AGATCCTAGCG	TAATTAC	ACTTGAGAATAGTTGGGGAGA

226/254

**FIGURE 46C (P2)**

CTAGACGTGGCTAGGATCGCATTAATGTGAACCTTATCAACCCCTCT  
 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT  
       GAAAGGTGACGTTAAGTCTCAGATTGGTCCTCGAACGATCAGGTTA  
 801 TCAACTGCAAAGACGTAATGGTCCAAATTCAAGTGTGACATGTGAGTA  
       AGTTGACGTTCTGCATTACCAAGGTTAACGTACACATGCTACACTCAT  
 851 TATTAATCCCTATCATAGCTCTCATGGGTATAGATGCGCACCTCCACCA  
       ATAATTAGGGATAGTATCGAGAGTACCCACATATCTACGCGTGGAGGTGGT  
 901 TCGTCACAGTTCTTGCTTGGCATTGCTGTTCTGGTAATTAAATGC  
       AGCAGTGTAAAAGAACGAAACGTAACGACAAGGACCATTAAAATTACG  
 951 TGATGTTGTATGGATCCTGAGCCCATACTGCGTATCGTAGGTCGAAATG  
       ACTACAAACATACTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAAACGGAAACGCAATA  
       CAGATACACAACATACTACAAATCCCTACCTTCTAAGGTGTTGCCTTGCCTAT  
 1051 CAGTTGTGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
       GTCAACACCGGTACGTTACGATTATGTCTACGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
       CTTTCTCTGTATGATAAGCTAGATTACCTTACAAATTGATGAATGC  
 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAAACTGCTGCA  
       CCATGTCAGGCCCTCAGATAACACTACTAGATACTAACGTTATGACGACGT  
 1201 ACTGATGCCACCGCTGGCAAATATGGGATAATGGAACCATCATAAAATCC  
       TGACTACGGTGGCGACCGTTATACCTTATTACCTTGGTAGTATTAGG  
 1251 CAGATCTAGTCTAGTTTAGCAGCGACATCAGGGAACAGTGGTACCAACAC  
       GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTCACCATGGTGTG  
 1301 TTACAGTGCACCAACACATTATGCCGTTAGTCAGGTTGGCTTCTACT  
       AATGTCACGTTGGTTGAAATACGGCAATCAGTTCCAACCGAACGGATGA  
 1351 AATAATACACAAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
       TTATTATGTGTTGGAAAACAATGTTGGTACAACACCGATATACCAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGA  
       AAACGTTCGTTATCACCTGTTACACCTATCTCCGACATCGTCACCTT  
 1451 AGGCTGAACAAACAGTGGCTCTTATGCAGATGGTCAATAACGTCCTCAG  
       TCCGACTTGTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCTTACAAGTGAATTCTAATACGGAAACAGT

227/254

**FIGURE 46C (P3)**

GTTTGGCTCTATTAACGGAATGTTCACTAAGATTATGCCCTTGTC  
1551 TGTTAACGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
ACAATTCTAGGAGAGAACACCAGGACGTAGGAGACCGGTTGCTACCTACA  
1601 TCAAGAACATGGAACCATTTAAATTGTATAGTGGATTGGTGTAGAT  
AGTTCTTACTACCTGGTAAAATTAAACATATCACCTAACCAACAACTCA  
1651 GTGAGGCATCGGATCCGAGCCTTAAACAAATCATTCTTACCCCTCTCCA  
CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
1701 TGGTGACCCAAACCAAATATGGTTACCATTTGATAGACAGATTACT  
ACCACTGGGTTGGTTACCAATGGTAATAAAACTATCTGTCTAATGA  
1751 CTCTTGCAGTGTGTGTGCCTGCCATGAAAATAGATGGCTAAATAAAAA  
GAGAACGTACACACACACAGGACGGTACTTTATCTACCGAATTATTTT  
1801 GGACATTGTAATTTGTAACGTAAAGGACAGCAAGTTATATCGAATTCC  
CCTGTAACATTAAAACATTGACTTCCCTGTCGTTCAATATAGCTTAAGG  
1851 TGCAG  
ACGTC

Total number of bases is: 1855.

Sequence name: PAP294

228/254

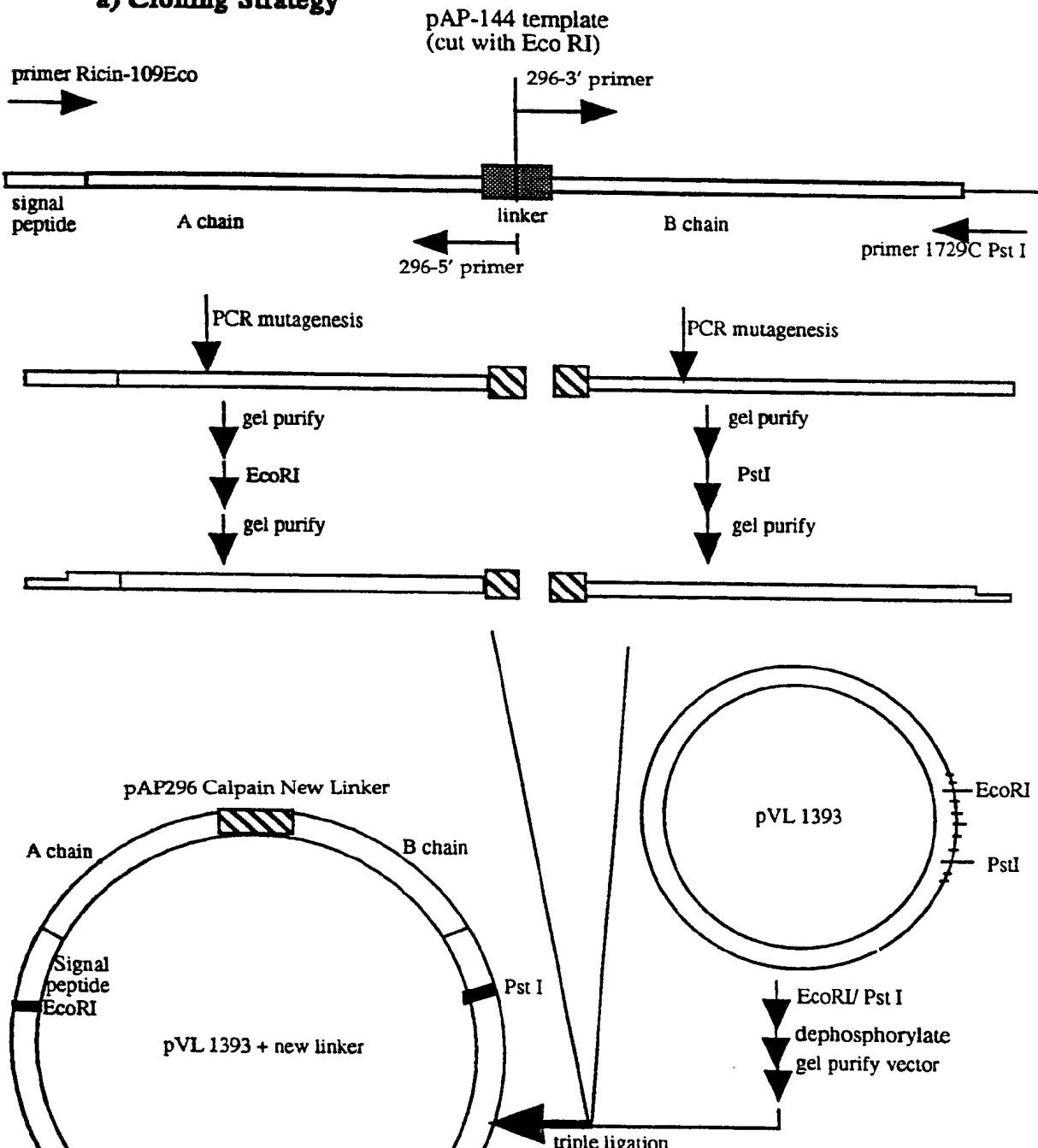
**FIGURE 46D**

Amino acid sequence Comparison of Mutant Preproricin Linker  
region of Neutrophil elastase to Wild Type

Wild type ricin linker:      A chain- S L L I R P V V P N F N -B chain

pAP-294 (Neutrophil elastase) linker:  
A chain- S L L G I A V P G N F N -B chain

229 / 254

**FIGURE 47A****PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393****a) Cloning Strategy**

230/254

FIGURE 47B

## Sequence of Calpain Linker Region

## WT preprocin linker

primer 296-3'  
5' - ACTCCTAGAACCCCCCCAGCTGATGTTGT - 3'  
\*\*\*\*\*  
-----  
TCTTGCTTATAAGGCCA|GTGGTACCAATTAAAT-----  
-----AGAAACGAATATTCCGGT|CACCATGGTTAAAATTA-----  
\* \*\*\* \* \* \*\*\*\*\*  
3' - AGCAGTGTCAAAAAAAAGTTTTATAACAA - 5'  
primer 296-5'

1) PCR mutagenesis

2) Ligate with pVL1393

pAP 296 linker  
(Calpain variant)

-----  
TTTTCAAAAATATTGTT|ACTCCTAGAACCCCCCCAA -----  
-----AAAAAGTTTTATAACAA|TGAGGGATCTGGGGGGGT

231/254

**FIGURE 47C (P1)**

Sequence of pAP296 insert

10	20	30	40	50
1 GAATTCATGAAACGGGAGGAAATACTATTGTAATATGGATGTATGCAGT				
CTTAAGTACTTGGCCCTCCTTATGATAACATTACACATACGTCA				
51 GGCAACATGGCTTGTTGGATCCACCTCAGGGTGGCTTCACATTAG				
CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAAATC				
101 AGGATAACAACATATTCCCCAACAAATAACCAATTATAAACTTACCA				
TCCTATTGTTGTATAAGGGTTGTTATGGTTAATATTGAAATGGTGT				
151 GCGGGTGCCACTGTGCAAAGCTACACAAACTTATCAGAGCTGTTCGCGG				
CGCCCACGGTGACACGTTCGATGTGTTGAAATAGTCTCGACAAGCGCC				
201 TCGTTAACAACTGGAGCTGATGTGAGACATGATATAACCAGTGTGCCAA				
AGCAAATTGTTGACCTCGACTACACTCTGTACTATATGGTCACAACGGTT				
251 ACAGAGTTGGTTGCCTATAAACCAACGGTTATTTAGTTGAACCTCTCA				
TGTCTCAACCAAACGGATATTGGTTGCCAAATAAAATCAACTTGAGAGT				
301 AATCATGCAGAGCTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA				
TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGGGTTACGTAT				
351 TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTCTTCATCCTGACA				
ACACCAGCCGATGGCACGACCTTATCGCGTATAAGAAAGTAGGACTGT				
401 ATCAGGAAGATGCAGAAGCAATCACTCATCTTCACTGATGTTAAAAT				
TAGTCCTTCTACGTCTCGTTAGTAGAAAGTGAACACTACAAGTTTA				
451 CGATATACATTGCCTTGGTGGTAATTATGATAGACTTGAAACAACCTGC				
GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACCTGTTGAACG				
501 TGGTAATCTGAGAGAAAATATCGAGTTGGAAATGGTCCACTAGAGGGAGG				
ACCATTAGACTCTTTATAGCTCAACCTTACCAAGGTGATCTCCTCC				
551 CTATCTCAGCGCTTATTACAGTACTGGTGGCACTCAGCTTCCAAT				
GATAGAGTCGCAAATAATAATGTCATGACCACCGTGAGTCGAAGGGTTGA				
601 CTGGCTCGTCTTATAATTGATCAGGAAATGAGTTCAAGCAGCAAG				
GACCGAGCAAGGAAATATTAAACGTAGGTTACTAAAGTCTCGTGTTC				
651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA				
TAAGGTTATATAACTCCCTTTACCGTGCTCTAATCCATGTTGGCCT				
701 GATCTGCACCAAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGAGA				

232/254

**FIGURE 47C (P2)**

CTAGACGTGGCTAGGATCGCATTAATGTGAACCTTATCAACCCCCCTCT  
 751 CTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTGCTAGTCCAAT  
       GAAAGGTGACGTTAAGTTCTCAGATTGGTCCTCGAAACGATCAGGTTA  
 801 TCAACTGCAAAGACGTAATGGTCCAATTCAAGTGTGTACGATGTGAGTA  
       AGTGACGTTCTGCATTACCAAGGTTAAGTCACACATGCTACACTCAT  
 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAAGATGCGCACCTCCACCA  
       ATAATTAGGGATAGTATCGAGAGTACCAACATATCTACGGTGGAGGTGGT  
 901 TCGTCACAGTTTTTCAAAAATATTGTTACTCCTAGAACCCCCCCCAGC  
       AGCAGTGTCAAAAAAAAGTTTTATAACAATGAGGATCTGGGGGGTCG  
 951 TGATGTTGATGGATCCTGAGCCCATAGTGCATCGTAGGTCGAAATG  
       ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTAC  
 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA  
       CAGATAACAACTACAATCCCTACCTTCTAAGGTGTTGCCCTTGCCTTAT  
 1051 CAGTTGTGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT  
       GTCAACACCGGTACGTTAGATTATGTCAGTTAGTCGAGACCTGAAA  
 1101 GAAAAGAGACAATACTATTGATCTAATGGAAAGTGTAACTACTTACG  
       CTTTCTCTGTTATGATAAGCTAGATTACCTTCACAAATTGATGAATGC  
 1151 GGTACAGTCGGGAGTCTATGTGATGATCTATGATTGCAAAACTGCTGCA  
       CCATGTCAGGCCCTCAGATAACACTAGATAACGTTATGACGACGTT  
 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAATCC  
       TGACTACGGTGGCGACCGTTATACCCATTACCTTGGTAGTATTAGG  
 1251 CAGATCTAGCTAGTTAGCAGCGACATCAGGGAACAGTGGTACACAC  
       GTCTAGATCAGATCAAATCGTCGCTGTAGTCCCTGTCACCATGGTGT  
 1301 TTACAGTGCAAACCAACATTATGCCGTTAGTCAGGTTGGCTTCTACT  
       AATGTCACGTTGGTTGTAATACGGCAATCAGTTCCAACCGAAGGATGA  
 1351 AATAATACACAACCTTTGTTACAACCATTGTTGGCTATATGGTCTGTG  
       TTATTATGTTGGAAAACAATGTTGGTAACAACCCGATAACCAAGACAC  
 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA  
       GAACGTTCGTTATCACCTGTTACACTATCTCCTGACATCGTCACTTT  
 1451 AGGCTGAACAAACAGTGGCTCTTATGCAGATGGTCAATACGTCTCAG  
       TCCGACTTGGTGTACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC  
 1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATACGGAAACAGT

233/254

**FIGURE 47C (P3)**

GTTTGGCTCTATTAAACGGAATGTTCACTAAGATTATGCCCTTGTC  
1551 TGTTAACGATCCTCTCTGTGGCCCTGCATCCTCTGGCCAACGATGGATGT  
ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTACA  
1601 TCAAGAACATGATGGAACCATTAAATTTGATAGTGGATTGGTAGAT  
AGTTCTTACTACCTGGTAAAATTAAACATATCACCTAACCAACATCTA  
1651 GTGAGGCAGTCGGATCCGAGCCTAAACAAATCATTCTTACCCCTCTCCA  
CACTCCGCTAGCCTAGGCTCGGAATTGTTAGTAAGAAATGGGAGAGGT  
1701 TGGTACCCAAACCAAATATGGTACCATATTGATAGACAGATTACT  
ACCACTGGGTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA  
1751 CTCTTGCAGTGTGTGTGCCTGCCATGAAAAATAGATGGCTAAATAAAA  
GAGAACGTACACACACAGGACGGTACTTTATCTACCGAATTATTTT  
1801 GGACATTGTAATTTGTAAGTGAAGGACAGCAAGTTATATCGAATTCC  
CCTGTAACATTAAAACATTGACTTCTGTCGTTCAATATAGCTTAAGG  
1851 TGCAG  
ACGTC

Total number of bases is: 1855.

Sequence name: PAP296

234/254

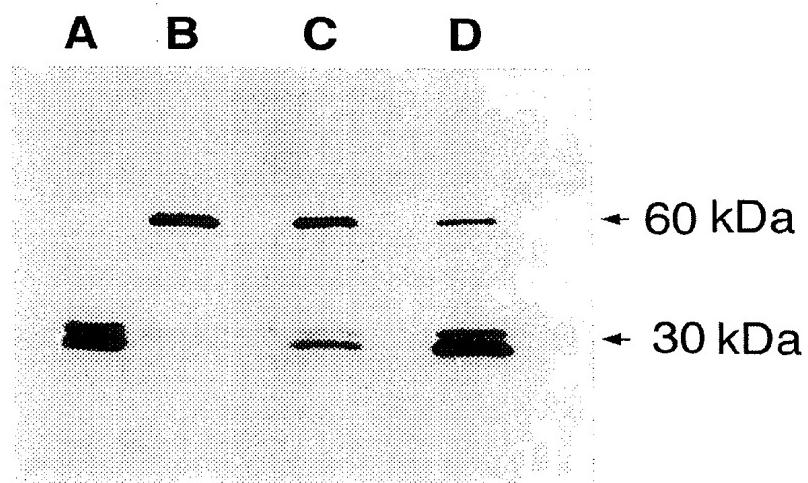
**FIGURE 47D**

**Amino acid sequence Comparison of Mutant Preproricin Linker  
region of Calpain to Wild Type**

**Wild type ricin linker:**      A chain- S L L I R P V V P N F N -B chain

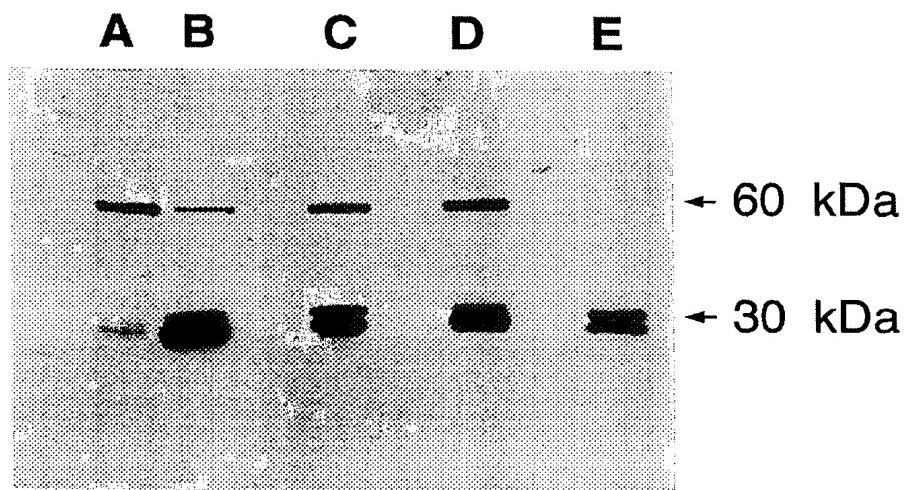
**pAP-296 (Calpain) linker:**      A chain- F F K N I V T P R T P P -B chain

235/254

**FIGURE 48****Cleavage of pAP 214 by Cathepsin B**

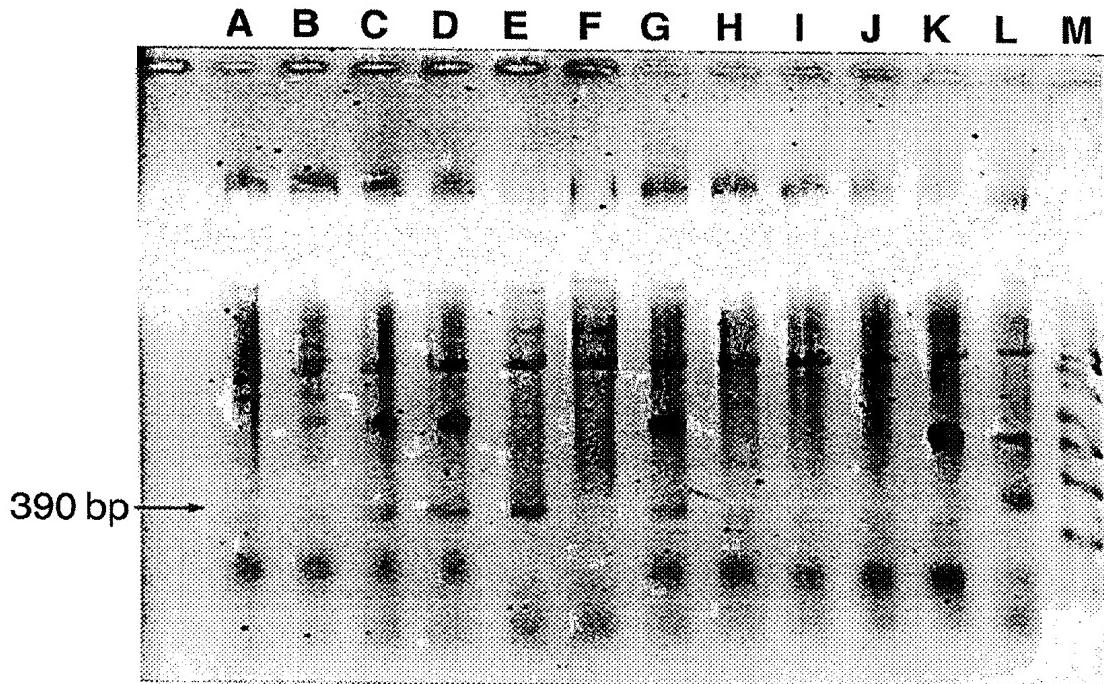
- A. Ricin standard**
- B. pAP 214**
- C. pAP 214 digested with 100 ng of Cathepsin B (18 hours)**
- D. pAP 214 digested with 618 ng of Cathepsin B (18 hours)**

236/254

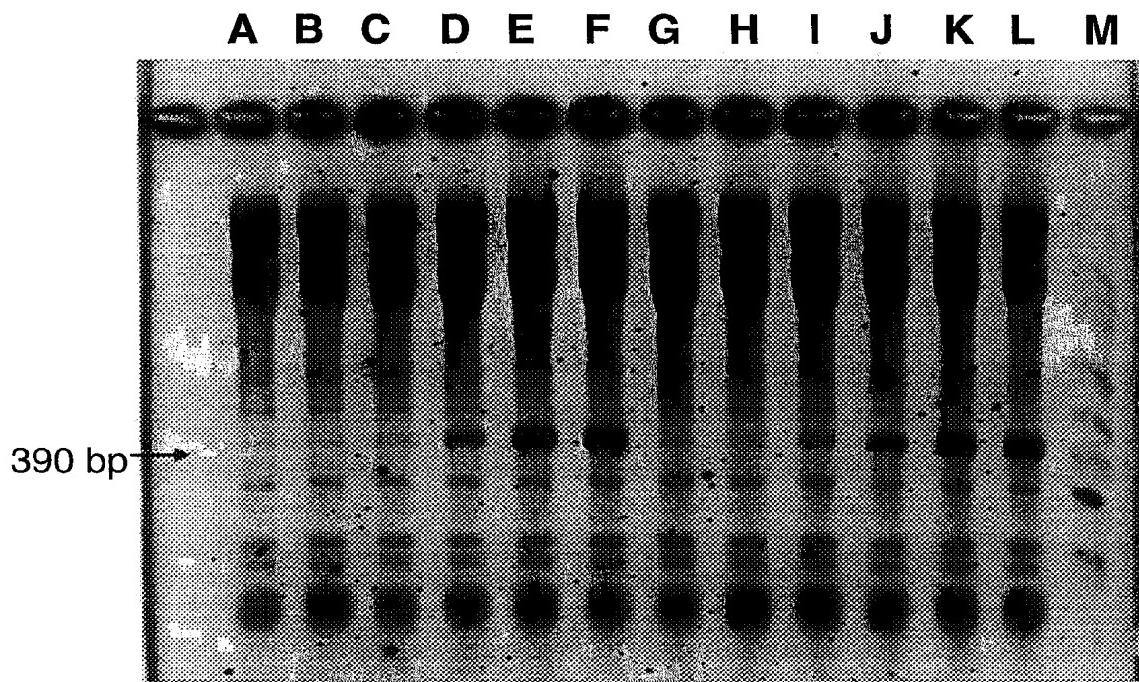
**FIGURE 49****Cleavage of pAP 220 with MMP-9**

- A. pAP 220
- B. pAP 220 digested with 200 ng of MMP-9 (16 hrs)
- C. pAP 220 digested with 20 ng of MMP-9 (16hrs)
- D. pAP 220 digested with 20 ng of MMP-9 (2hrs)

237/254

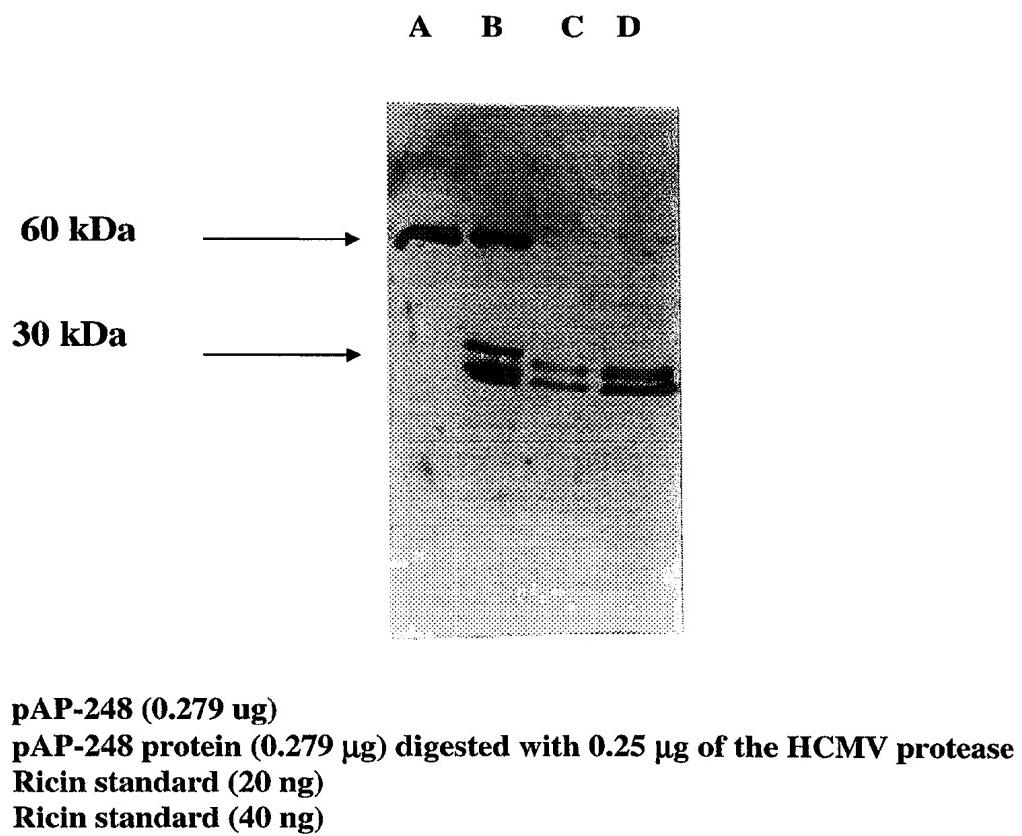
**FIGURE 50****Activation of pAP 214**

- A. 41.7 pg of pAP 214 digested with Cathepsin B
- B. 291 pg of pAP 214 digested with Cathepsin B
- C. 2.0 ng of pAP 214 digested with Cathepsin B
- D. 14.2 ng of pAP 214 digested with Cathepsin B
- E. 100 ng of pAP 214 digested with Cathepsin B
- F. Negative control
- G. Ricin A chain
- H. 41.7 pg of pAP 214 variant
- I. 291 pg of pAP 214 variant
- J. 2.0 ng of pAP 214 variant
- K. 14.2 ng of pAP 214 variant
- L. 100ng of pAP 214 variant
- M. RNA ladder

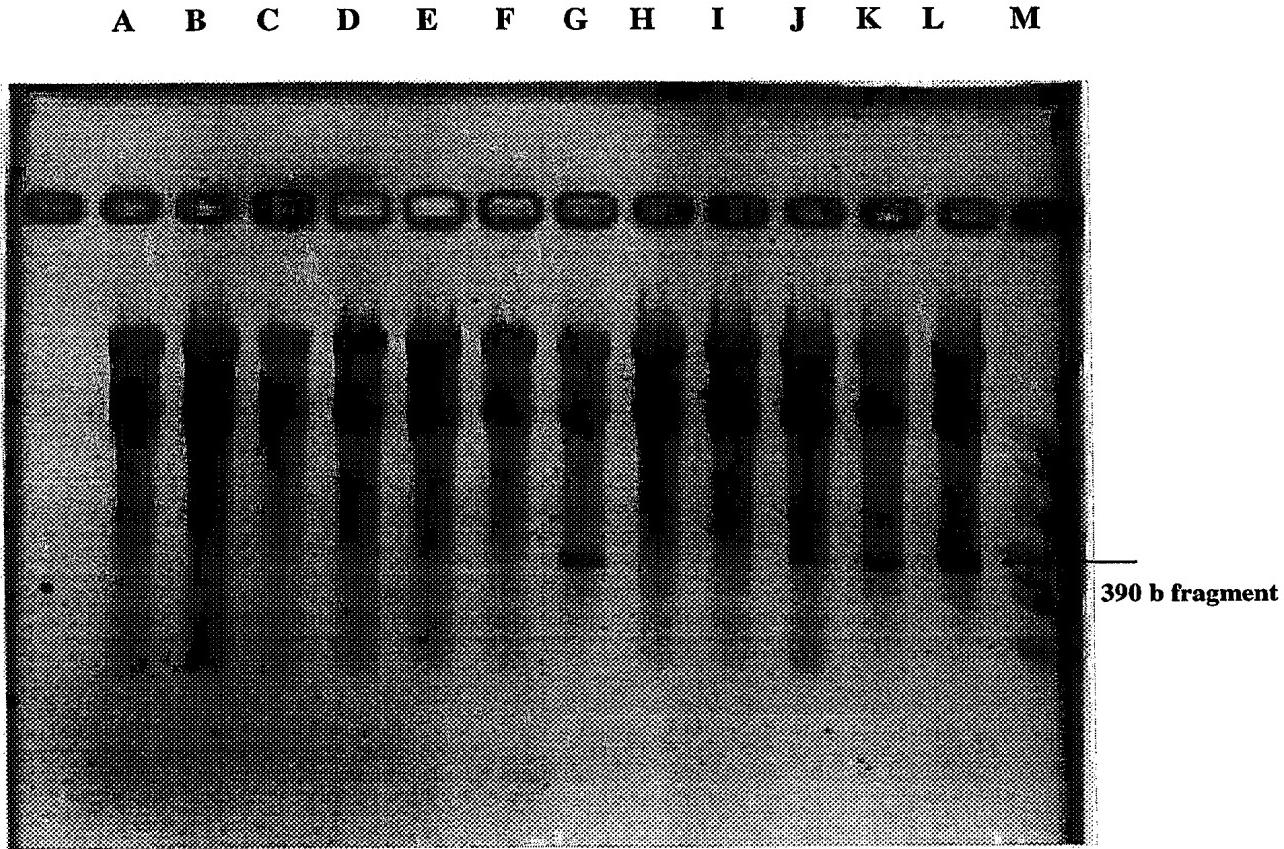
**FIGURE 51****Activation of pAP 220**

- A. 48.5 pg of pAP 220 variant
- B. 291 pg of pAP 220 variant
- C. 2.0 ng of pAP 220 variant
- D. 14.3 ng of pAP 220 variant
- E. 100 ng of pAP 220 variant
- F. Ricin A chain
- G. Negative Control
- H. 48.5 pg of pAP 220 variant digested with MMP-9
- I. 291 pg of pAP 220 variant digested with MMP-9
- J. 2.0 ng of pAP 220 variant digested with MMP-9
- K. 14.3 ng of pAP 220 variant digested with MMP-9
- L. 100 ng of pAP 220 variant digested with MMP-9
- M. RNA ladder

239/254

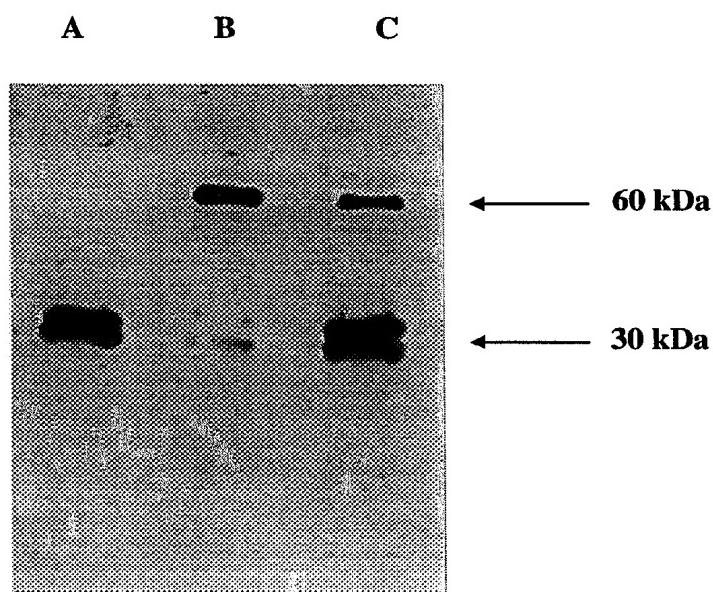
**FIGURE 52****Cleavage of pAP-248 Protein by The Human Cytomegalovirus (HCMV) protease**

240/254

**FIGURE 53****Activation of pAP-248 Protein**

- A. 90 ng of pAP-248 variant
- B. 12.8 ng of pAP-248 variant
- C. 1.8 ng of pAP-248 variant
- D. 260 pg pAP-248 variant
- E. 37 pg of pAP-248 variant
- F. Negative control
- G. Ricin A chain
- H. 37 pg of pAP-248 digested with HCMV protease
- I. 260 pg of pAP-248 digested with HCMV protease
- J. 1.8 ng of pAP-248 digested with HCMV protease
- K. 12.8 ng of pAP-248 digested with HCMV protease
- L. 90 ng of pAP-248 digested with HCMV protease
- M. RNA ladder

241/254

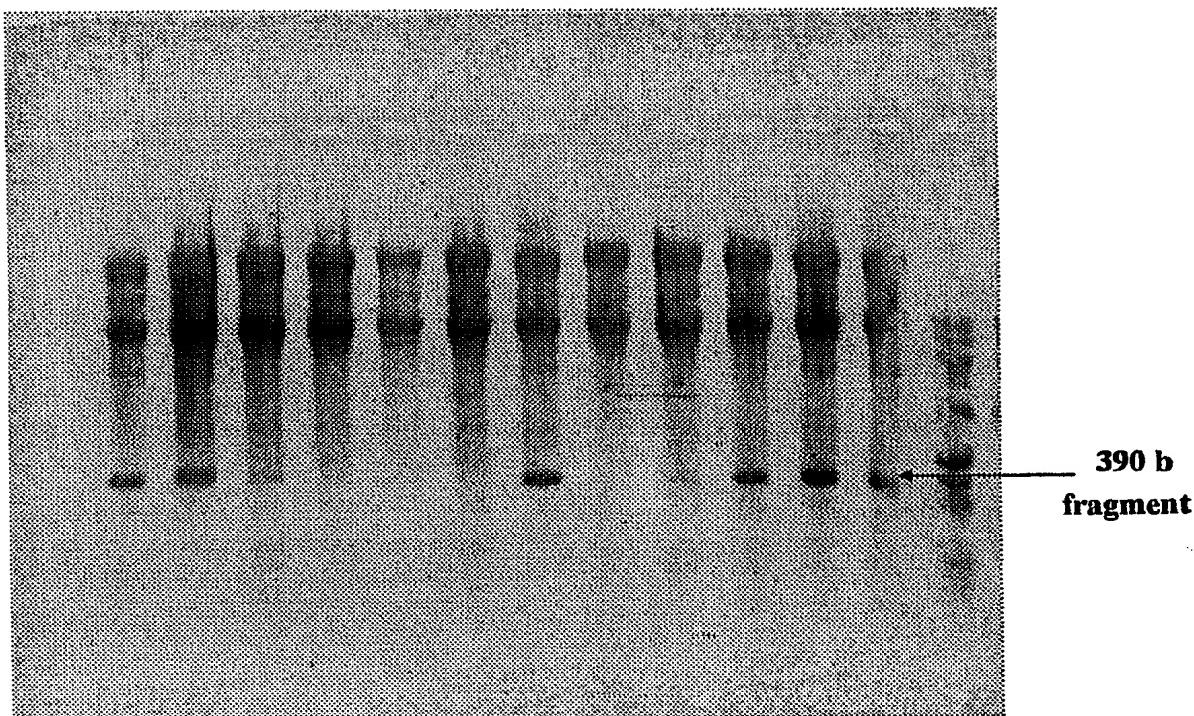
**FIGURE 54****Cleavage of pAP-256 protein by The Hepatitis A Virus 3C (HAV 3C) Protease**

- A. Ricin standard (0.250 µg)
- B. pAP-256 protein (0.378 µg)
- C. pAP-256 protein digested (0.302 µg) with 1.25 µg of the HAV 3C protease

242/254

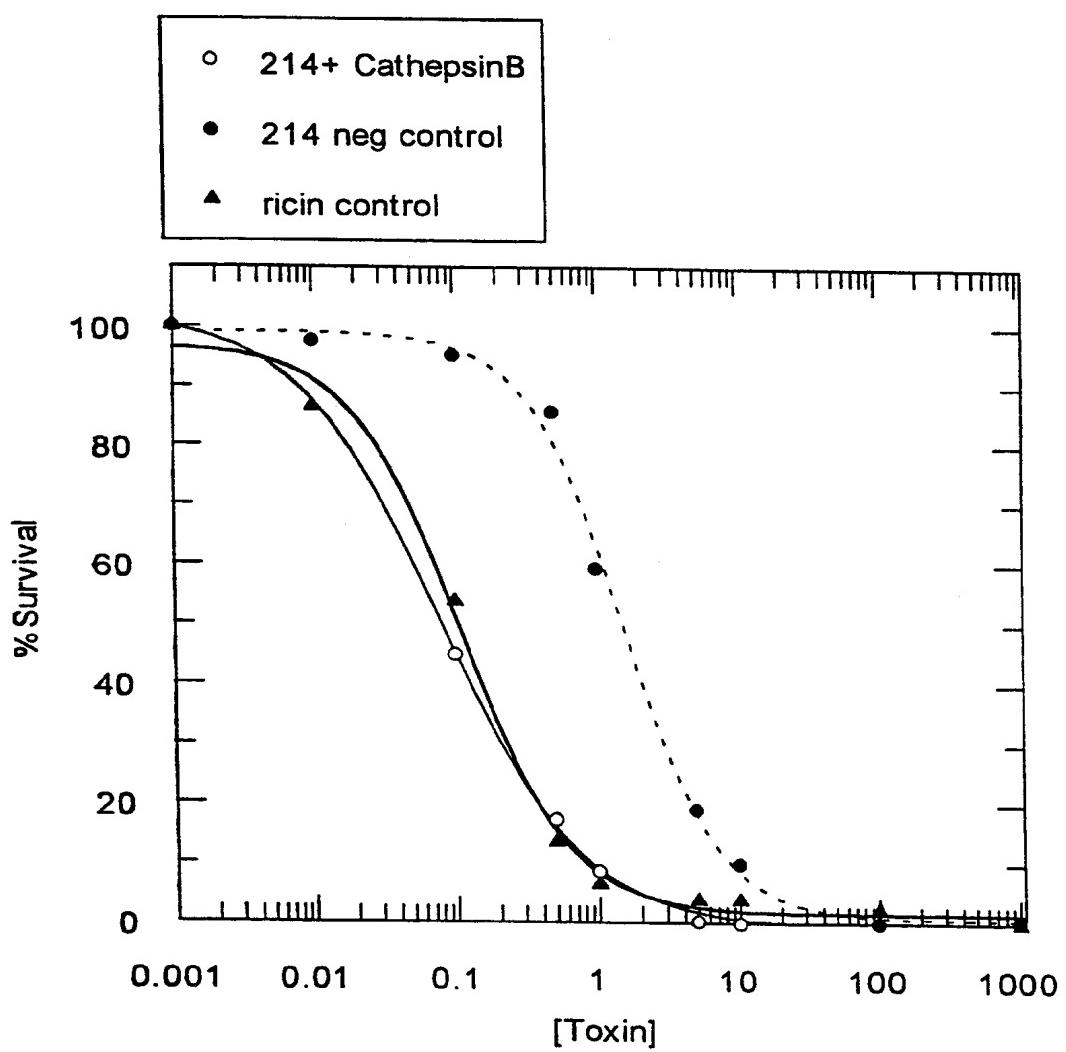
**FIGURE 55****Activation of pAP-256 Protein**

A B C D E F G H I J K L M



- A. 100 ng of pAP-256 variant
- B. 14.2 ng of pAP-256 variant
- C. 2.0 ng of pAP-256 variant
- D. 291 pg of pAP-256 variant
- E. 41.7 pg of pAP-256 variant
- F. Negative control
- G. Ricin A chain
- H. 41.7 pg of pAP-256 digested with HAV 3C protease
- I. 291 pg of pAP-256 digested with HAV 3C protease
- J. 2.0 ng of pAP-256 digested with HAV 3C protease
- K. 14.2 ng of pAP-256 digested with HAV 3C protease
- L. 100 ng of pAP-256 digested with HAV 3C protease
- M. RNA ladder

243/254

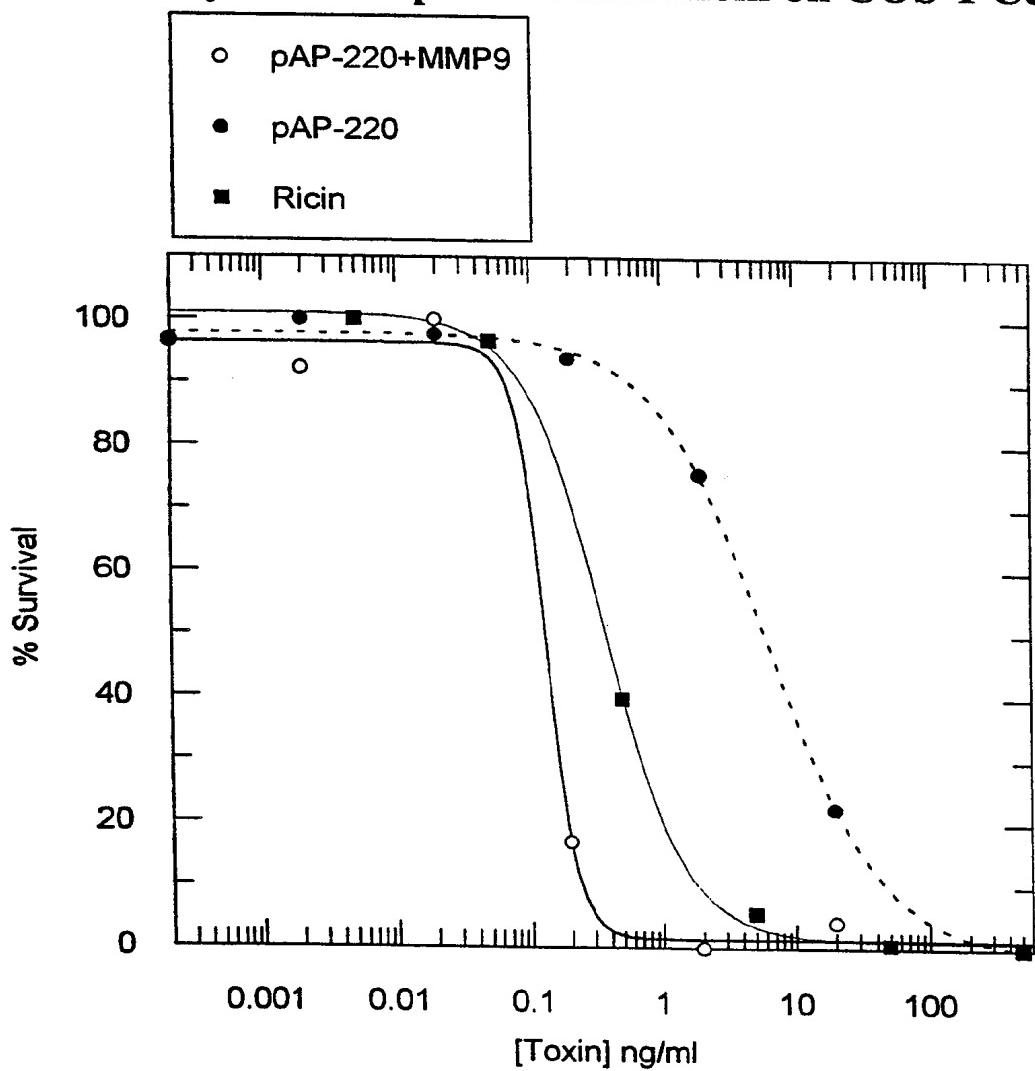
**FIGURE 56****Cytotoxicity of Digested and Undigested pAP 214 with Cathepsin B to COS-1 Cells**

	Ricin	pAP 214	pAP 214 + Cathepsin B
IC <sub>50</sub> (ng/ml)	0.11	1.9	0.078
Relative Toxicity	1X	17X	0.7X

244/254

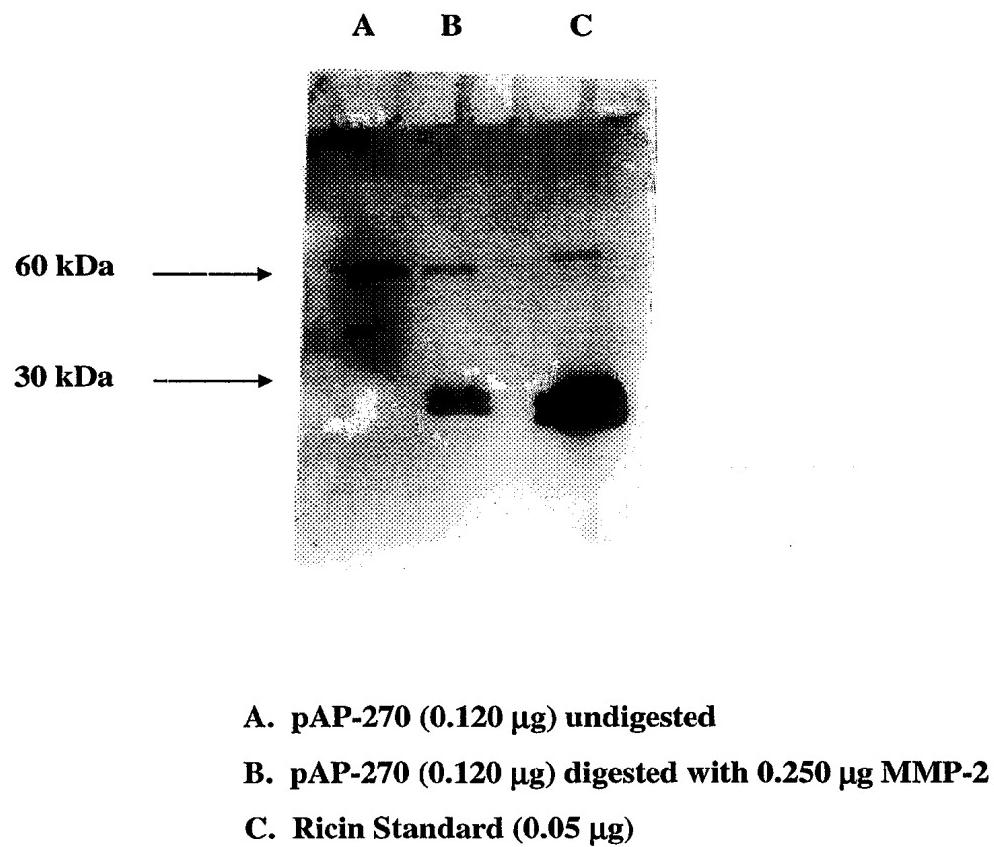
**FIGURE 57**

**Cytotoxicity of pAP220 Digested with MMP-9 Compared to Freshly Thawed pAP220 and Ricin on COS-1 Cells**



	Ricin	pAP 220	pAP 220 + MMP-9
IC <sub>50</sub> (ng/ml)	0.31	6.7	0.13
Relative Toxicity	1X	22X	0.4X

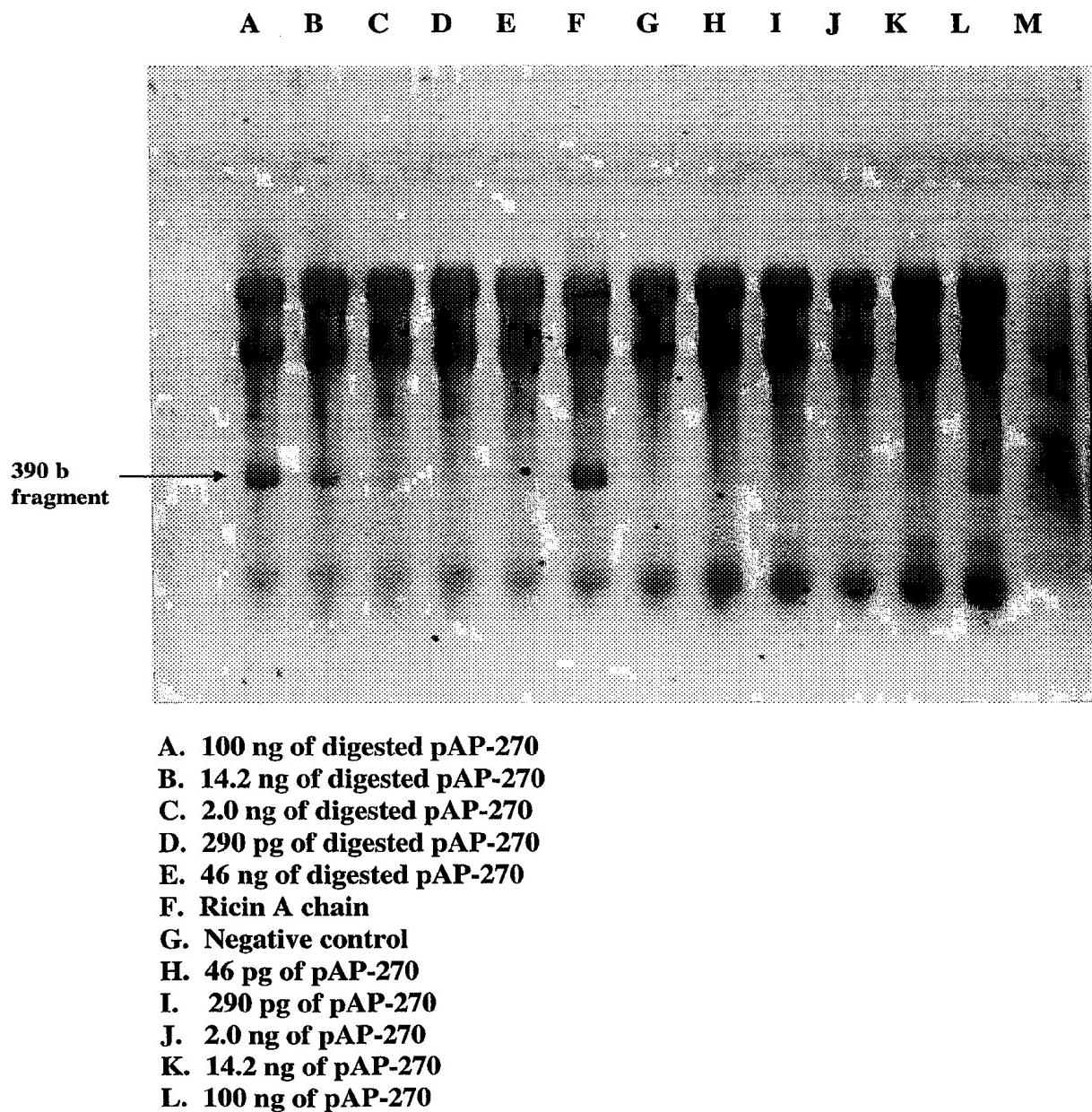
245/254

**FIGURE 58****Cleavage of pAP-270 protein by The Matrix Metalloproteinase 2 (MMP-2)**

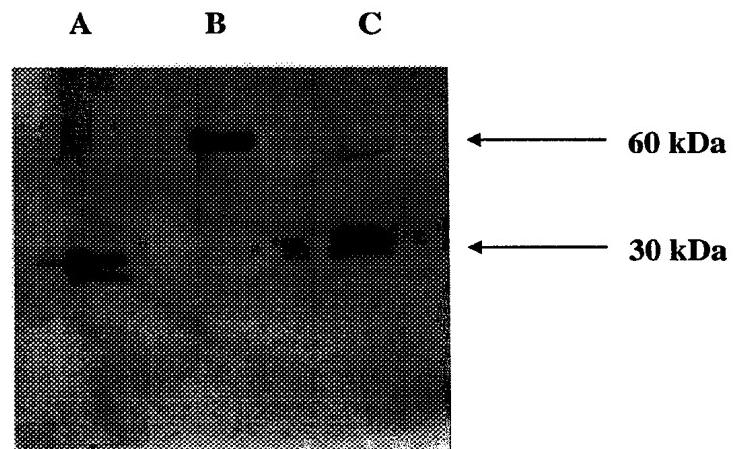
246 / 254

**FIGURE 59**

Activation of pAP-270 protein

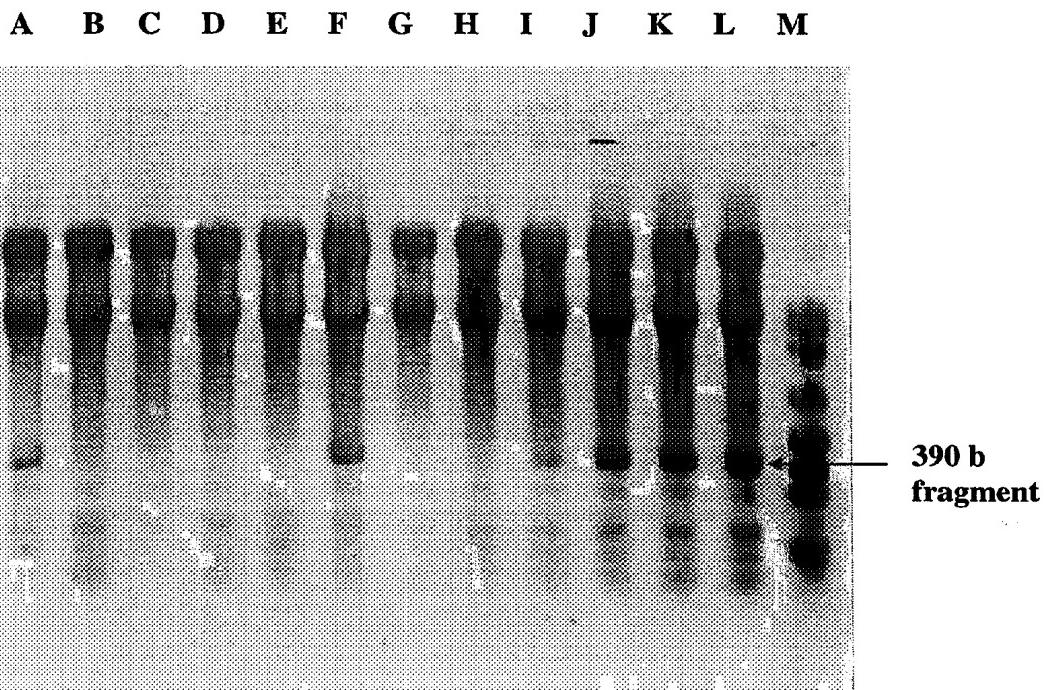


247/254

FIGURE 60**Cleavage of pAP-288 protein by Plasminogen Tissue Activator (t-PA)**

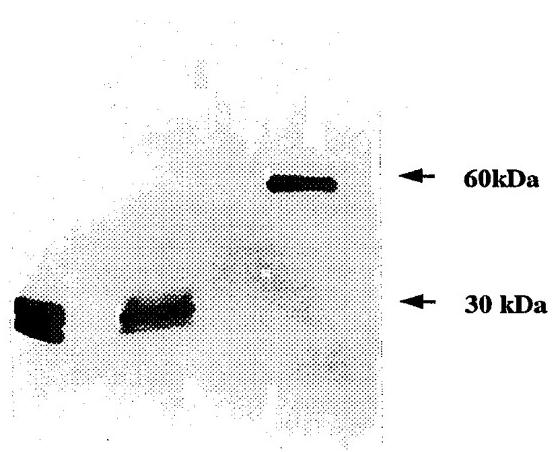
- A. Ricin Standard (0.05µg)
- B. pAP-288 (0.66 µg) undigested
- C. pAP-288 (0.60 µg) digested with 0.18 µg of t-PA protease

248/254

**FIGURE 61****Activation of pAP-288 protein**

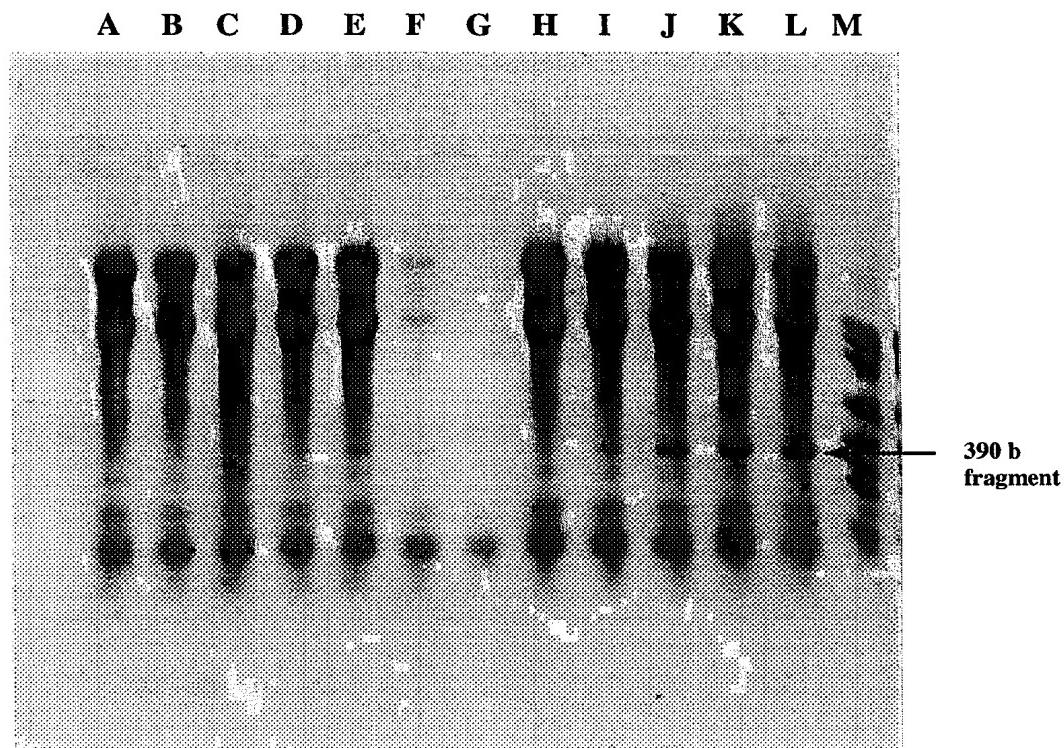
- A. 200 ng of pAP-288
- B. 28.4 ng of pAP-288
- C. 4.0 ng of pAP-288
- D. 482 pg of pAP-288
- E. 83.4 pg of pAP-288
- F. Ricin A chain
- G. Negative control
- H. 83.4 pg of pAP-288 digested with tissue Plasminogen Activator (t-PA)
- I. 482 pg of pAP-288 digested with t-PA
- J. 4.0 ng of pAP-288 digested with t-PA
- K. 28.4 ng of pAP-288 digested with t-PA
- L. 200 ng of pAP-288 digested with t-PA
- M. RNA ladder

249/254

FIGURE 62**Cleavage of pAP 294 With Human Neutrophil Elastase**

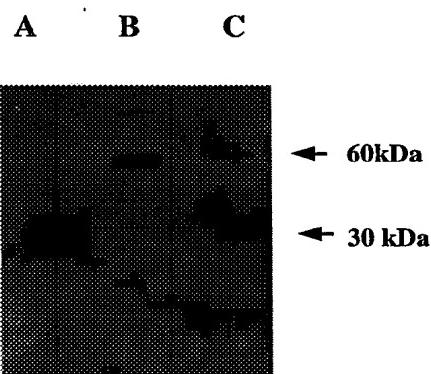
- A. Ricin Standard ( 0.050 µg)
- B. pAP 294 protein ( 0.171 µg) digested with 1.42 µg of Human Neutrophil Elastase
- C. pAP 294 protein ( 0.121 µg)

250/254

FIGURE 63**Activation of pAP 294 Protein**

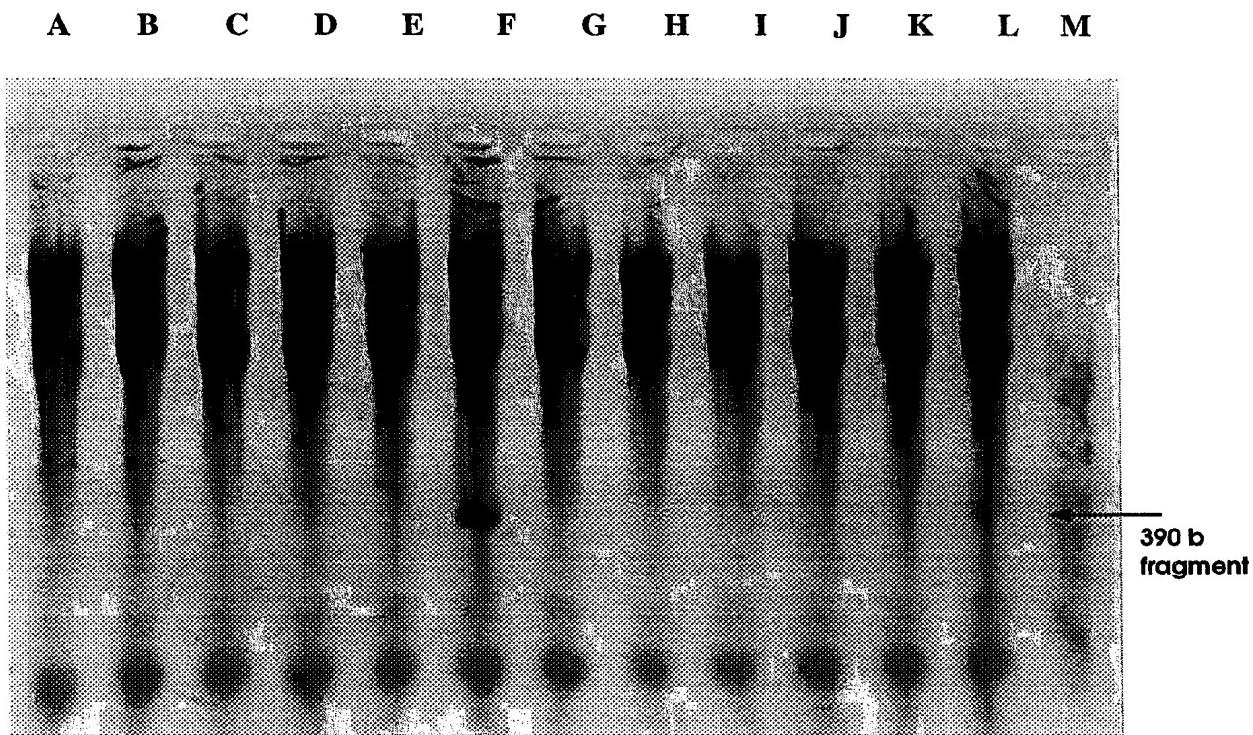
- A. 60 ng of pAP 294
- B. 8.57 ng of pAP 294
- C. 1.22 ng of pAP 294
- D. 175 pg of pAP 294
- E. 25 pg of pAP 294
- F. Ricin A chain
- G. Negative Control
- H. 360 ng of pAP 294 digested with Human Neutrophil Elastase
- I. 51 ng of pAP 294 digested with Human Neutrophil Elastase
- J. 7.3 ng of pAP 294 digested with Human Neutrophil Elastase
- K. 1.0 ng of pAP 294 digested with Human Neutrophil Elastase
- L. 150 pg of pAP 294 digested with Human Neutrophil Elastase
- M. RNA ladder

251/254

**FIGURE 64****Cleavage of pAP 296 with Calpain**

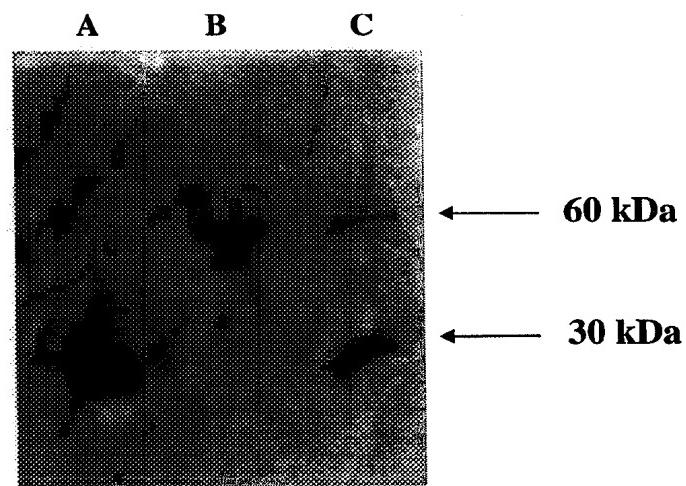
- A. Ricin Standard (0.05 µg)
- B. pAP 296 (0.761 µg) undigested
- C. pAP 296 (0.761 µg ) digested with 4.0 µg of Calpain

252/254

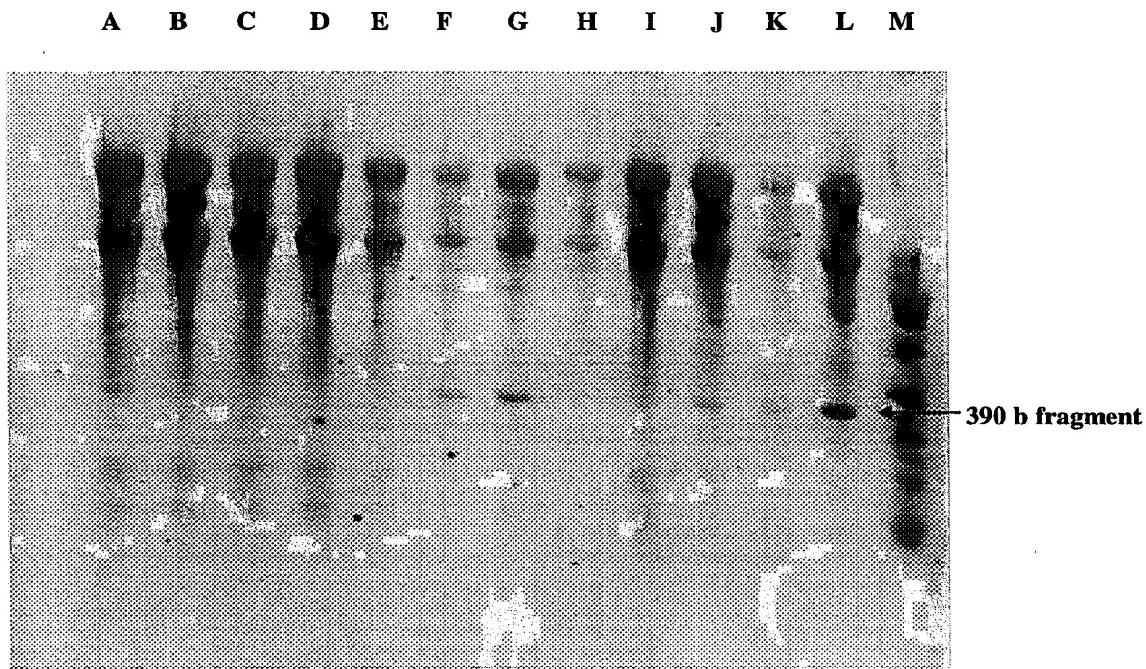
**FIGURE 65****Activation of pAP 296 Protein**

- A. 100 ng of pAP 296 variant
- B. 14.2 ng of pAP 296 variant
- C. 2.0 ng of pAP 296 variant
- D. 290 pg of pAP 296 variant
- E. 46 pg of pAP 296 variant
- F. Ricin A chain
- G. Negative control
- H. 46 pg of pAP 296 variant digested with Calpain
- I. 290 pg of pAP 296 variant digested with Calpain
- J. 2.0 ng of pAP 296 variant digested with Calpain
- K. 14.2 ng of pAP 296 variant digested with Calpain
- L. 100 ng of pAP 296 variant digested with Calpain
- M. RNA ladder

253/254

**FIGURE 66****Cleavage of pAP-222 Protein by The Matrix Metalloproteinase 2 (MMP-2)**

- A. Ricin Standard (0.250 ug)
- B. pAP-222 Protein (0.250 ug)
- C. pAP-222 protein ( 0.250 ug) digested with 0.28 ug of MMP-2

**FIGURE 67****Activation of pAP-222 Protein**

- A. 100 ng of pAP-222 variant
- B. 14.2 ng of pAP-222 variant
- C. 2.0 ng of pAP-222 variant
- D. 291 pg of pAP-222 variant
- E. 41.7 pg of pAP-222 variant
- F. Ricin A chain
- G. Ricin A chain
- H. 41.7 pg of pAP-222 digested with MMP-2
- I. 291 pg of pAP-222 digested with MMP-2
- J. 2.0 ng of pAP-222 digested with MMP-2
- K. 14.2 ng of pAP-222 digested with MMP-2
- L. 100 ng of pAP-222 digested with MMP-2
- M. RNA ladder